Fruit fly (Diptera: Tephritidae) ecology in the Western Cape, South Africa

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DECLARATION

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Abstract

The Mediterranean fruit fly (medfly) *Ceratitis capitata* (Wiedemann) and the Natal fly *C. rosa* Karsch are two important tephritid pest species of commercially grown deciduous fruit in the Western Cape. Detailed information on the status of these two fruit fly species in terms of the influence that different host fruits have on their development, abundance and distribution in this region is not known. This project investigated the status of the two fruit fly species in the Western Cape by a) determining the influence of different fruit types on life table parameters such as egg development, larval development, pupal success rate, adult emergence, fecundity and adult survival, b) assessing trapping data and fruit infestation from home gardens in or near areas where deciduous fruits are grown commercially, in order to determine relative abundances and distribution of *C. capitata* and *C. rosa* in the region, and c) using geometric morphometrics to assess variability in development associated with host type, by determining shape variation in the wings of *C. capitata* that were reared on different host fruits. Life table parameters of *C. capitata* and *C. rosa* were determined with a series of laboratory experiments on "Golden delicious" and "Granny smith" apples (*Malus domestica* L. Borkh.), "Crimson seedless" and "Dauphine" grapes (*Vitis vinifera* L.), "Excellence" peaches (*Prunus persica* Sieb. & Zucc.), "Packham's triumph" pears (*Pyrus communis* L.), "Angeleno" plums (*Prunus japonica* Thunb.), "Navel" oranges (*Citrus sinensis* Osbeck), clementine (*Citrus unshiu* Swingle) and "Fan Retief" guava (*Psidium guajava* Linn.). To gain a broader understanding of the population dynamics of fruit flies, on different commercial and non-commercial host plants in various fruit growing areas during different months of the year, baited traps were installed and fruit infestation of known and potential host fruits were assessed at selected sites. Geometric morphometrics were used to assess shape variation of wings of a F1 generation of *C. capitata* reared on different host fruits, namely plum, pear and clementine that were of the same varieties as mentioned above.

No significant differences ($p = 0.773$) were found in egg hatch between fruit fly species on the different deciduous fruit types grown commercially in the Western Cape: grape, plum, pear, apple and peach. No positive puncture response was found on oranges, therefore this fruit type was excluded from further analyses. *Ceratitis capitata* and *C. rosa* favoured guavas and displayed significant preferences for this fruit in terms of field collected samples and developmental parameters. Developmental success was significantly higher on guavas compared to other fruit types tested, for males and females ($p < 0.015$), of both *C. capitata* and *C. rosa*. Piquanté peppers (*Capsicum baccatum* L.) and jambos (*Syzygium jambos* (L.) Alston) were also significant alternate host plants based on high fruit infestation rates in the field and they should be the focus of control actions in home gardens. Patterns of relative abundance of the two fruit fly species were found to adhere to seasonality in terms of host availability and certain abiotic factors such as annual rainfall and elevation. *Ceratitis capitata* was found to be the dominant species, as has been recorded previously in other studies. Significant differences were found in the wing shape of males and females of *C. capitata* only. Shape variation was significant for flies reared on different fruit types, more so for males. These results suggest developmental differences for flies reared on different hosts. Results of the present study can be used to gain a better understanding of factors that determine the relative distribution of these two species and which hosts they more readily infest in the Western Cape.

Key words: Fruit flies, host, abundance, distribution, development

Opsomming

Die Mediterreense vrugte vlieg (medvlieg) *Ceratitis capitata* (Wiedemann) en die Natal vlieg *C. rosa* Karsch is twee belangrike tephritid pes spesies van kommersiële bladwisselende vrugte in die Wes-Kaap. In diepte inligting oor die status van hierdie twee vrugte vlieg spesies in terme van die invloed wat verskillende gasheer vrugte op die ontwikkeling, volopheid en verspreiding van hierdie vlieë in die Wes-Kaap het, is nie bekend nie. Die huidige studie ondersoek die status van hierdie vrugte vlieg spesies in die Wes-Kaap deur a) vas te stel wat die invloed is wat verskillende vrugte op lewens tabel parameters het soos eier ontwikkeling, larwe ontwikkeling, papie sukses, volwasse opkoms, vrugbaarheid en volwasse oorlewing, b) deur gebruik te maak van lokval data en besmette vrugte vanaf tuine van huise, wat naby of in areas voorkom waar vrugte kommersieel gegroei word, sodat daar vasgestel kan word wat die relatiewe volopheid en verspreiding van *C. capitata* en *C. rosa* in hierdie streek is, en c) om geometriese morfometriese metodes te gebruik om vas te stel wat die verskille in die ontwikkeling van die vrugte vlieë is wanneer hul op verskillende tipes gasheer vrugte geteel word, deur te kyk na verskille in die vorme van die vlerke. Lewens tabel parameters was vasgestel vir “Golden delicious” en “Granny smith” appels (*Malus domestica* L. Borkh.), “Crimson seedless” en “Dauphine” druiwe (*Vitis vinifera* L.), “Excellence” perskes (*Prunus persica* Sieb. & Zucc.), “Packham’s triumph” pere (*Pyrus communis* L.), “Angeleno” pruime (*Prunus japonica* Thunb.), “Navel” lemoene (*Citrus sinensis* Osbeck), naartjies (*Citrus unshiu* Swingle) and “Fan Retief” koejawels (*Psidium guajava* Linn.) deur ‘n reeks van laboratorium eksperimente.

Lokvalle was uitgesit en vrugte was versamel in die tuine van huise wat in of naby areas is waar vrugte kommersieel gegroei word, om ‘n breër kennis te verkry van die dinamika agter die vrugte vlieg populasies op verskillende kommersiële en nie-kommersiële gasheer plante, tydens verskillende maande van die jaar. Geometriese morfometriek was gebruik om die

verskille in die vorme van die vlerke van ‘n F1 generasie *C. capitata* wat op verskillende gasheer vrugte geteel was te bepaal, naamlik pruime, pere en naartjies. Hierdie vrugte was dieselfde variëteite as die bogenoemde vrugte.

Daar was geen aansienlike verskille ($p = 0.773$) in die uitbroei van eiers, tussen die vrugte vlieë spesies, wat uit verskillende gasheer vrugte wat kommersieel in die Wes-Kaap gegroei word (druiwe, pruime, pere, appels en perskes) versamel was nie. Geen steekmerke was waargeneem op lemoene nie en om hierdie rede is geen verdere analises op lemoene gedoen nie. *Ceratitis capitata* en *C. rosa* het koejawels verkies en het aansienlike voorkeur vir dìe vrug betoon in terme van vrugte wat in die veld versamel was, en ontwikkelings parameters. Die ontwikkelings sukses was aansienlik hoër vir koejawels teenoor ander vrugte wat getoets was vir beide mannetjies en wyfies ($p < 0.015$) van *C. capitata* en *C. rosa*. Piquanté rissies (*Capsicum baccatum* L.) en jambos (*Syzygium jambos* (L.) Alston) was aansienlike alternatiewe gasheer plante wat tydens veldwerk versamel was en moet die fokuspunt vorm van beheer aksies in huise se tuine. Patrone in die relatiewe vespreiding van die vlieë is beïnvloed deur seisonale patrone in terme van die beskikbaarheid van gasheer plante, en sommige abiotiese faktore soos reënval en hoogte bo seespieël. *Ceratitis capitata* was die dominante spesie, soos al reeds voorheen vasgestel was tydens ander studies. Aansienlike verskille is waargeneem in die vorme van die vlerke van slegs *C. capitata* mannetjies en wyfies. Daar was aansienlike variasie in vlerk vorme vir vlieë wat op verskillende vrugte tipes geteel was, meer so vir mannetjies vlieë. Die resultate dui daarop dat verskille in die ontwikkeling van vlieë voorkom wat op verskillende gasheer vrugte geteel word. Die resultate van die huidige studie kan dus help om beter te verstaan watter faktore die relatiewe volopheid en verspreiding van hierdie vrugte vlieë beïnvloed. Dit kan ook help om die tipe gashere wat meer geredelik aangeval word beter te bepaal vir die Wes-Kaap.

Sleutelwoorde: Vrugte vlieë, gasheer, volopheid, verspreiding, ontwikkeling

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Chapter 1: General introduction

Study area

The Western Cape Province is located in South Africa and is the focus area of this study. It is situated at the southern tip of the African continent and is surrounded by the Atlantic Ocean on the west coast and the Indian Ocean on the east coast. It has a Mediterranean-type climate with cold, wet winters and hot, dry summers and is therefore situated in a winter rainfall region. The Western Cape has average annual minimum and maximum temperatures of 10.77°C and 22.89°C, respectively, and average annual minimum and maximum rainfalls of 165.33mm and 959.34mm, respectively (ARC, 2014). It is a mountainous region (Cape Fold Mountains) with elevations ranging from sea-level to 2325m. The adequate rainfall and climatic conditions in the Western Cape enable stable annual production of deciduous fruit, namely stone (apricots, peaches, plums and nectarines) and pome (apples and pears) fruit, table grapes, wine grapes as well as soft citrus (Vink & Tregurtha, 2004; National Agricultural Marketing Council (NAMC), 2007). The region is a centre for commercially-grown deciduous fruits, and the majority (74%) of South Africa's fruit production areas are located here (Deciduous Fruit Producers' Trust (DFPT), 2005).

Large percentages of the fruits produced in the region are exported to overseas countries. In 2013, South Africa produced 1.612 million tons of apples, pears, apricots, peaches, nectarines and plums (Hortgro, 2013) of which 0.71 million tons (44.04%) were exported, generating gross export earnings of approximately R7.02 billion (Hortgro, 2013). South Africa is the 13th overall producer of apples and the 9th overall producer of pears in the world (Hortgro, 2013). Due to the large scale of commercial fruit production in the Western Cape, it is important to focus on the cost effective management of insect pests of the fruits produced in the province.

Insect pests cause major economic losses of commercial crops. In the Western Cape fruit production and export is negatively influenced by two economically important fruit fly species, namely, the Mediterranean fruit fly (medfly) *Ceratitis capitata* (Wiedemann) and the Natal fruit fly *C. rosa* Karsch (Diptera: Tephritidae) (Barnes et al., 2007, De Villiers et al., 2013), which are both quarantine pests (Barnes et al., 2007). This status limits export of fruits produced in areas where these flies have become established into countries with quarantine restrictions or regulations. An importing country with quarantine regulations against these fruit flies could deny an exporting country a potential market for their fruit, which in turn would lead to the exporting country having to introduce expensive disinfestation treatments before the fruit can be considered safe for export (Christenson & Foote, 1960; White & Elson-Harris, 1992).

Study species

The family Tephritidae, true fruit flies, is a large family with about 4000 species in 500 genera and is distributed throughout the temperate, subtropical and tropical regions of the world (Christenson & Foote, 1960; White & Elson-Harris, 1992; De Meyer et al., 2008). Of the 4000 species, about 100 have been reported to be economically important (White & Elson-Harris, 1992). They occur in all fruit growing areas of the world and are destructive insect pests of fruits, nuts and vegetables (Christenson & Foote, 1960; White & Elson-Harris, 1992). The genus *Ceratitis* consists of more than 90 species which are all native to the Afrotropical region (De Meyer et al., 2008). *Ceratitis capitata* has a worldwide distribution through the international movement of fruit (De Meyer, 1999), while *C. rosa* is currently limited to sub-Saharan Africa (White & Elson-Harris, 1992). These two economically important fruit fly pests continuously threaten to invade new areas through numerous morphological, physiological and behavioural adaptations which allow them to thrive in

diverse habitats (Yuval & Hendrichs, 1999), and are both genetically and ecologically closely related (De Meyer et al., 2008; Duyck et al., 2010).

Ceratitis capitata has been recognized as the most economically important insect pest of fruits in the world, and has been reported to utilize over 370 plant species (Copeland et al., 2002) and more than 250 types of fruit that are commercially grown (Fimiani, 1989). *Ceratitis rosa* has been reported to utilize 107 plant species (De Meyer et al., 2002). In the Western Cape, *C. capitata* is known to infest 48 plant species and *C. rosa* 30 plant species (Myburgh, 1956; Manrakhan & Addison, 2014).

The behaviour of adult *C. capitata* flies has been described as being extremely complex due to their wide range of host preference and their need to move through heterogeneous environments during their search for carbohydrate and protein food sources, mates and oviposition sites (Yuval & Hendrichs, 1999).

Biology

Upon eclosion from the puparium, the newly emerged adult fruit fly must make its way to the surface of the soil, through the use of peristaltic movements, after which it begins its adult life. The adult stage of both species is similar, with *C. rosa* being slightly larger (Blomefield et al., 2015). Male and female fruit flies both require carbohydrate and protein rich diets, with females utilizing the nutrients for, primarily, egg production and males for energy consuming mating rituals (Yuval & Hendrichs, 1999). The adults are small, about 3mm – 5mm (*C. capitata*) and 8mm (*C. rosa*) in length, and are yellowish-orange with a brown tinge and are characterized by patterned bands on their wings with black, brown and brownish-yellow markings (Blomefield et al., 2015). *Ceratitis rosa* is distinguishable from *C. capitata* by the patterns on the scutellum and by characteristic ornamentation on the legs of male *C. rosa*,

which is lacking in *C. capitata* males. The latter are diagnosable through the lower orbital bristle, which is spatulate (White & Elson-Harris, 1992). Adults take ten days to reach sexual maturity (Papadopoulos et al., 2002) and, under field conditions, could live for two to three months (Christenson & Foote, 1960). Adults have been reported to live up to six months under favourable conditions (Blomefield et al., 2015). They have multivoltine generations, facilitated by the availability of fruit on different host plant species at different times of year (De Meyer, 2001).

Females of both species of fruit fly have a long extendable ovipositor that is used for laying eggs into the fleshy seed bearing organs of host plants (White & Elson-Harris, 1992). Adult females lay their eggs in the flesh under the skin of intact, or detached, usually ripe fruits and vegetables which will serve as food for the developing larval stages. Eggs are white, smooth and banana-shaped, 1mm in length (Blomefield et al., 2015) and take between two and four days to hatch after which the larvae immediately start feeding in the fruit (Christenson & Foote, 1960). Females lay one to ten eggs per oviposition cavity, but 50 or more eggs can be found per cavity, originating from multiple females (Blomefield et al., 2015). It has been observed that *C. capitata* females, during oviposition, may mark the fruit with a deterring pheromone that cause other females from this species to not attack the same fruit (White & Elson-Harris, 1992), which in turn leads to high fruit infestation rates if these pest species are not controlled. Ovipositing directly damages the fruit which affects the outside appearance and attractiveness of the fruit and larvae feeding inside the fruit render the fruit unsuitable for human consumption and export.

Larvae undergo three instars inside the host fruit, taking between 10 and 45 days (Blomefield et al., 2015), before exiting the fruit to pupate in the soil (Christenson & Foote, 1960; Tsitsipis, 1989). Krainacker et al. (1987) found that the average development time for larvae of *C. capitata* was between seven and twelve days at 30°C. At the end of the third instar the

larvae exit the fruit to pupate in the soil. These larvae can jump relatively great distances of up to 22.86cm (Christenson & Foote, 1960) in their search for an optimal pupation site (White & Elson-Harris, 1992). Jumping from the fruit also serves as a mechanism for reducing the larvae's chances of encountering predators upon exiting the fruit (Maitland, 1992; Yuval & Hendrichs, 1999). After dropping to the ground the larvae bore into the soil to a depth of between 8.89cm and 13.97cm, for *C. capitata* and *C. rosa*, respectively (Myburgh, 1956), where they form puparia in which the larvae could take between six and eleven days (Christenson & Foote, 1960) to complete metamorphosis. The puparia are cylindrical with rounded ends and are between 4mm and 6mm in length (Blomefield et al., 2015). After complete metamorphosis adults eclose from the pupae and have to find their way back up to the soil surface, completing the life cycle.

Host plants

A host plant is described as a plant on which the animal completes its normal development in nature (Aluja & Mangan, 2008). After fruit fly eggs hatch inside the host fruit the larvae start feeding. This constitutes tunneling inside the fruits, which make the fruit unsuitable for human consumption, and has economic impacts on the farmers of such host fruits.

From a biological perspective, for fruit-feeding insects like fruit flies, a host is a fruit or vegetable species (irrespective of its stage of maturity or previous damage) in which the insect can complete its development from egg to pupa or adult (immature development or complete immature and mature development). From an applied perspective, a host plant is more restricted and depends on the maturity stage of the host fruit or vegetable and prior damage to the host by other organisms (Cowley et al., 1992; Aluja & Mangan, 2008).

The acceptance or rejection of a plant by an insect is a behavioural response to certain physical or chemical features of the plant which provide visual and chemical cues to the insect (Bernays & Chapman, 1994). During host selection, the insect has to differentiate between the quality and quantity of stimuli originating from a potential host plant. This includes differentiating between wavelengths of light and types of chemicals being emitted by the plant, and is achieved by the insect by having cells which are sensitive to different modalities (Bernays & Chapman, 1994).

All plants release volatiles, which are linked to the process of water loss that occurs through the stomatal opening. These volatiles can move great distances away from the plant, given the right conditions, and are sometimes characteristic to certain plants (Bernays & Chapman, 1994). Insects are attracted to or repelled from the plant by these volatiles, due to specific preferences or prerequisites for a host plant. Secondary metabolites of plants are considered a main factor that either attract or repel insects (Jermy, 1984). Bernays & Chapman (1994) mention that no two plant species have the same profile of secondary metabolites, and are chemically different. Furthermore, the nutritional levels in plants are always in a state of flux. The quality and quantity of compounds within a plant species are different and this is furthermore affected by variable environmental factors that influence the plant's chemistry (Bernays & Chapman, 1994). It was found that for the Mexican fruit fly, *Anastrepha ludens* (Loew), and for the Ethiopian fruit fly, *Dacus ciliatus* Loew, that a mixture of plant volatiles from host fruits were more attractive than the individual components (Robacker et al., 1992; Alagarmalai et al., 2009), but ripe fruit elicited a greater response (Alagarmalai et al., 2009).

Host plant selection involves choosing the right individual plant, within a species, in which feeding, survival and development can occur (Bernays & Chapman, 1994). For fruit fly females the selection process consists of positively responding to a range of stimuli from the host plant, such as finding the host, accepting the host and ovipositing in the host. Host plant

selection by insects is primarily relient on chemoreception, and the development of a new insect/host plant relationship is thus a combination of the evolutionary changes in the insect's chemosensory system and the evolution of plant chemistry (Jermy, 1984). The insect's adaptation to the nutritional quality of the new host plant is thus a secondary process (Jermy, 1984). This makes determining a set range of host plants for *C. capitata* and *C. rosa*, which are polyphagous generalists, an ongoing process as their host selection may change over time. This will in turn affect the temporal range and distribution of these fruit fly species, according to the range and availability of suitable host plants.

Fruit fly management

Areas infested by fruit flies should be managed in order to prevent population numbers from increasing to the point where total crop losses would occur (Rössler et al., 2000; Blomefield et al., 2015). Various control methods exist for controlling and monitoring fruit fly numbers. These include lure-baited traps, control of host plants (removal or fruit-stripping), sanitation, application of fruit fly bait, bait stations, classical biological control through augmentative releases of parasitoids, and the sterile insect technique (Blomefield et al., 2015). It has been recommended that the SIT, integrated with other methods, represents the most effective method of controlling fruit flies in a South African context (Barnes et al., 2007).

Fruit fly pests in South Africa are mainly controlled by the bait application technique (BAT) in the form of sprays or bait stations (Barnes, 2000). BAT is where a protein attractant is mixed with an insecticide in order to attract and kill targeted fruit fly pest species (Rössler, 1989) and is usually the first control measure taken in the event of a fruit fly outbreak (White & Elson-Harris, 1992). The control method is based on the principle that fruit fly males and females are attracted to protein sources, such as protein hydrolysates, that release ammonia as

a by-product of their breakdown (White & Elson-Harris, 1992; Mazor et al., 2002; Mazor, 2009). BAT is a more localized method of control compared to full cover sprays of insecticides only. The protein bait is typically mixed with an organophosphate such as malathion, (Wood & Harris, 1989) also known as maldison or mercaptothion, which is then sprayed directly onto the crop in a grid-like fashion (with sites of application typically 15m apart) throughout the orchard (White & Elson-Harris, 1992). During times of peak activity this procedure is repeated on a weekly basis. The BAT in the form of baited sprays could negatively affect non-target species when conventional insecticides are used (Leza et al., 2008). BAT can also be applied in an orchard in the form of a bait station such as the M3 bait station (Ware et al., 2003). This is a ready-to-use attract and kill station which consists of a protein hydrolysate attractant and a toxicant (Ware et al., 2003). Bait stations exclude the risk of pesticidal residues landing on fruits and negatively affecting non-target species, as is the case with spraying techniques.

More recently, an already formulated protein bait containing spinosad (a naturally derived insecticide from actinomycete soil organisms) (GF-120, Dow AgroSciences Southern Africa (Pty) Ltd., Bryanston, South Africa) was developed and found to be a suitable alternative to malathion based bait sprays, as it displayed significant levels of *C. capitata* control and is environmentally safer than malathion (Peck & McQuate, 2000; Chueca et al., 2007), as it rapidly degrades in the environment (Burns et al., 2001). Burns et al. (2001) found similar results and reported that spinosad reduced *C. capitata* populations by 80%, with no adverse effects on honey bee broods or significant changes in the hive condition. The spinosad based bait GF-120 was also found to have no effect on beneficial insects in citrus orchards and on fruit fly parasitoids (Vargas et al., 2001; Thomas & Mangan, 2005).

A few agricultural areas of the Western Cape make use of the Sterile Insect Technique (SIT) that targets only *C. capitata* (Manrakhan & Addison, 2014). In SIT using *C. capitata*, a

genetic sexing strain is used for the rearing of only male flies. With such a strain females have a temperature sensitive lethal mutation which allows for the elimination of females by killing the embryos through a heat treatment of the fruit fly eggs (Barnes et al., 2004). The male larvae are provided with food and water until they develop to the pupal stage during which they are exposed to gamma radiation (Barnes et al., 2004; Barnes & Venter, 2008). The gamma radiation damages the males' sex chromosomes and the males that emerge from irradiated pupae are sterile. The sterile males compete with wild males to mate with wild females, which in turn produce sterile eggs, due to the damaged male sex chromosomes that disrupt the normal development of the embryo, which subsequently then result in a population decline (Knipling, 1955). Sterile males are released through aerial or ground releases of between 2000 and 5000 flies per hectare per week in commercial agricultural areas where fruit flies are prevalent and pose phytosanitary risks to the crops produced (Barnes & Venter, 2008). The number of sterile flies released during SIT should be determined according to the level of fruit fly abundance in the area. During the pilot SIT project of the Hex River Valley, sterile flies were released in numbers ranging from 2000/ha/week in the town of De Doorns, where the main crop was table grapes, to 5000/ha/week in rural gardens and hotspots in the surrounding area (Barnes & Venter, 2008). This method was evaluated some years later and found to be less effective, in areas with high population pressure, compared to areas treated with bait applications, and where the method was not applied on an area-wide basis (Manrakhan & Addison, 2014).

The application of supplementary methods are often used in combination with SIT, as these methods, such as BAT reduce the number of wild population males that sterile males have to compete with during SIT releases (FAO, 2007). Depending on the degree to which BAT or SIT is implemented, these techniques could be used for managing population numbers or eradicating pests from agricultural areas. These techniques could be applied in conjunction

with one another on an area-wide scale, as opposed to treating only hotspots with one or the other technique (Manrakhan & Addison, 2014). Sanitation is also recommended as an essential part of fruit fly management, where all infested fruits are removed from or under host plants once a week (Grout & Moore, 2015). These fruits should then be disposed of by burying it at a depth of at least 60cm or by processing the fruit through a hammer mill (Grové et al., 2015). Grové et al. (2015) also recommend removing any alternate host plants that occur in fruit production areas as they serve as a breeding ground and source of the fruit flies.

The co-existence of the two fruit fly species in only a limited number of fruit production areas of the Western Cape reflects the importance of understanding the relative abundance and distribution of *C. capitata* and *C. rosa*, as influenced by biotic factors, in particular host availability, host preference and interspecific competition, and abiotic factors such as temperature, rainfall and elevation (Duyck et al., 2008). It is important to focus monitoring efforts on home gardens and farm gardens as these act as reservoirs for the fruit flies when preferred commercial crop host fruits are not available, allowing them to breed throughout the year (De Villiers et al., 2013).

Post-harvest treatment

Fruits produced in areas where either *C. capitata* or *C. rosa* have become established, have to undergo post-harvest disinfestation if these fruits are to be exported to countries that are under strict quarantine regulations (Christenson & Foote, 1960). Harvested fruit are treated before export using techniques such as fumigation (using methyl bromide), hot water vapour or hot water heat treatment, cold treatments, insecticide dipping or irradiation (White & Elson-Harris, 1992). The Western Cape produces 74% of South Africa's deciduous fruit. The top export markets of deciduous fruits that are commercially produced in South Africa are

briefly listed here with the percentages of the total exports for the specific fruits, exported to each region. The United Kingdom (apples 27%, pears 12%, apricots 21%, peaches 36%, nectarines 47%, plums 25%), the Far East & Asia (apples 20%, pears 11%), Europe & Russia (apples 14%, pears 60%, apricots 47%, peaches 13%, nectarines 19%, plums 52%) and the Middle East (pears 11%, apricots 31%, peaches 43%, nectarines 27%, plums 16%) (Hortgro, 2013).

The Western Cape is an example of an area of commercial fruit production where *C. capitata* and *C. rosa* have become established. In order to keep markets open for export it is necessary to treat fruits before they are exported, according to specific treatment schedules for specified pests of certain fruits, as established by the importing countries. Procedures are agreed upon and enforced by the Department of Agriculture, Forestry and Fisheries of South Africa (DAFF). For example, apples being exported to Taiwan have to be treated against *C. capitata* with one of the following schedules: Cold treatment between 12 days at 0°C or 18 days at 3.33°C, or fumigation using 32g/m^3 methyl bromide at 21°C for between two and three hours (DAFF, 2014). Apples and pears being exported to the United States must be cold treated for 14 days at 1.11°C or below, and peaches, plums, nectarines and grapes have to be cold treated for 22 days at -0.55°C against known fruit fly species of South Africa (USDA, 2007). Similar treatment schedules are in place for exporting citrus and grapes to China, grapes and persimmons to Israel, citrus to Japan and Korea, apples and pears to Mexico and citrus to the USA (DAFF, 2014). Disinfestation treatments are expensive (Christenson & Foote, 1960) and should be avoided through better managing fruit pests such as *C. capitata* and *C. rosa*. Quarantine procedures are designed for relieving propagule pressure of undesirable pest species, and quarantine methods aimed at *C. capitata* will also be effective against other fruit fly species with a similar host range, such as *C. rosa* (De Meyer et al., 2008).

Current gaps in knowledge

In order to optimise pre- and post harvest treatments of the two fruit fly pest species *C. capitata* and *C. rosa*, a better understanding of their relative abudance in different areas and hosts as well as utilization of hosts by these two species is required. It is currently unknown how well *C. capitata* and *C. rosa* develop in host plants that they have been recorded on. It is also unclear whether the known distribution of the two fruit fly species in the Western Cape is a true reflection of their abundances or simply a lure response. The list of known preferred or alternate host plants of these two fruit flies is insufficient and outdated. It is therefore necessary to establish a comprehensive list of all host plant species that are accepted by both *C. capitata* and *C. rosa* in the Western Cape. Co-infestation experiments, for the two species of fruit flies in different host plants, should be carried out in conjunction with the host plant acceptance monitoring in order to determine the degree of interspecific competition, during larval stages, which will enable a better understanding of how the two species of fruit flies coexist in different areas.

Aims and objectives

Aluja & Mangan (2008) recommend extensive field sampling to establish natural infestations and associations with natural hosts as a first step to determining whether a fruit or vegetable is a natural or non-host for a specific fruit fly species. This has not been done recently to any great extent in South Africa. The present study aims to provide fruit fly management programmes with quantitative data on relative abundance of the two fruit fly pest species and utilization of hosts, which can be used to refine the implementation of management actions against fruit fly pests in the Western Cape. The objectives, to attain the above aims, were as follows:

a) To assess trapping data and infested fruits from home gardens, in or near areas where deciduous fruits are grown commercially, in order to determine relative abundances and distribution of *C. capitata* and *C. rosa* in the Western Cape.

b) To assess different developmental parameters of *C. capitata* and *C. rosa* on different host fruits. The influence that the fruit type has on life table parameters such as egg development, larval development, pupal success rate (the number of larvae that successfully developed into pupae), adult emergence, fecundity and adult survival was determined.

c) To measure wing shape as a means of assessing variability of development in specific host fruits.

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Chapter 2: Relative abundance of two fruit fly species, *Ceratitis capitata* (Wiedemann) and *Ceratitis rosa* Karsch (Diptera: Tephritidae), on non-commercial hosts in the Western Cape Province, South Africa

Introduction

Ceratitis capitata and *C. rosa* (Diptera: Tephritidae) are two fruit fly pest species that occur in many areas of the Western Cape Province in South Africa (Myburgh, 1956; De Villiers et al., 2013). *Ceratitis capitata* was found to be the predominant fruit fly pest species in all regions of the Western Cape (Manrakhan & Addison, 2014). While *C. capitata* was recorded on a large range of commercial and non-commercial hosts, *C. rosa* was found to be more limited in its host choices (Myburgh, 1956; Manrakhan & Addison, 2014). *Ceratitis capitata* has a significantly higher critical thermal maximum than *C. rosa* (Nyamukondiwa & Terblanche, 2009), which further aids in explaining the observed distribution and abundance of the two fly species in the Western Cape.

Fruit flies are controlled primarily with the Bait Application Technique (BAT), utilizing protein hydrolyzate baits mixed with organophosphate insecticides or spinosad (GF-120, Dow AgroSciences, Southern Africa (Pty. Ltd.)), full cover insecticide applications (Barnes, 2000) or M3 bait stations (Ware et al., 2003). The Sterile Insect Technique (SIT) has also been operational against *C. capitata* since 1999. Control using the area-wide principle is only applied with BAT, whereby helicopters are used for application over entire production areas (Baard, 2014a; Baard, 2014b). SIT is applied only in hotspots through ground releases (Manrakhan & Addison, 2014). For this reason, it is critical to know the role that alternate hosts play and which hosts require targeted fruit fly management.

Studying the spatio-temporal distribution of flies within an agroecosystem can provide improved management recommendations for monitoring efforts and reduce management costs (Papadopoulos et al., 2003). It has been further observed that hotspots of *C. capitata* in Italy can be related to not only the type of host fruit, but also to the stage of development (maturation) and fruit availability on the tree and on the ground (Sciaretta & Trematerra, 2011). If an annual pattern in distribution of certain pest species can be quantified, farmers would greatly benefit from such data as management practices, such as labour-intensive sanitation measures, can then be fine-tuned and ultimately result in the lower input of pesticides into agro-ecosystems. An important requirement for management techniques of *C. capitata* and *C. rosa* is to determine the composition of these two species in terms of adult population and fruit infestation, in different fruit growing areas of the Western Cape (Manrakhan & Addison, 2007). This has been quantified to some extent in commercial orchards and non-commercial hosts in the Western Cape (De Villiers et al., 2013), but due to the variety of different non-commercial hosts that have not been sampled, it is important to gather more information for host utilization by fruit flies in home gardens. The aim of this chapter is therefore to establish the population dynamics of *C. capitata* and *C. rosa* in home gardens associated with commercial areas in order to quantify the role of alternate hosts more clearly in Western Cape fruit production areas. To further improve monitoring efforts, the influence of different lures was assessed for the monitoring of these two fruit fly species.

Materials and methods

Five non-commercial sites (home gardens) in the Western Cape Province were selected for sampling, based on their close proximity to areas of commercially-grown deciduous fruits (Fig. 2.1). The five sites were located in Stellenbosch, Elgin, Villiersdorp and Worcester: The

sites were as follows: Stellenbosch University Botanical Gardens (S 33˚56'10.99" E 18˚51'56.186") (Altitude = 121m), Welgeleë Lodge gardens in Stellenbosch (S 33˚59'21.9" E 18˚45'49.117") (Altitude 36m), Bon Acres home garden in Elgin (S 34˚8'24.586" E 19˚1'32.318") (Altitude = 319m), a home garden in the town of Villiersdorp (S 33˚59'34.573" E 19˚17'38.598") (Altitude = 345m) and Summerhill guest farm gardens in Worcester (S 33˚36'5.407" E 19˚25'24.095") (Altitude = 347m). Data collected from the two sites in Stellenbosch have been combined and from here on represent "Stellenbosch", unless stated otherwise. The sites are situated in an area that experiences a mean annual rainfall of between 165.3 and 959.34mm, and mean temperatures ranging between 10.77 and 22.89°C (ARC, 2014). Data for this chapter were obtained using traps and collected fruits from the sites to monitor adult fruit fly abundance and distribution for specific times of the year.

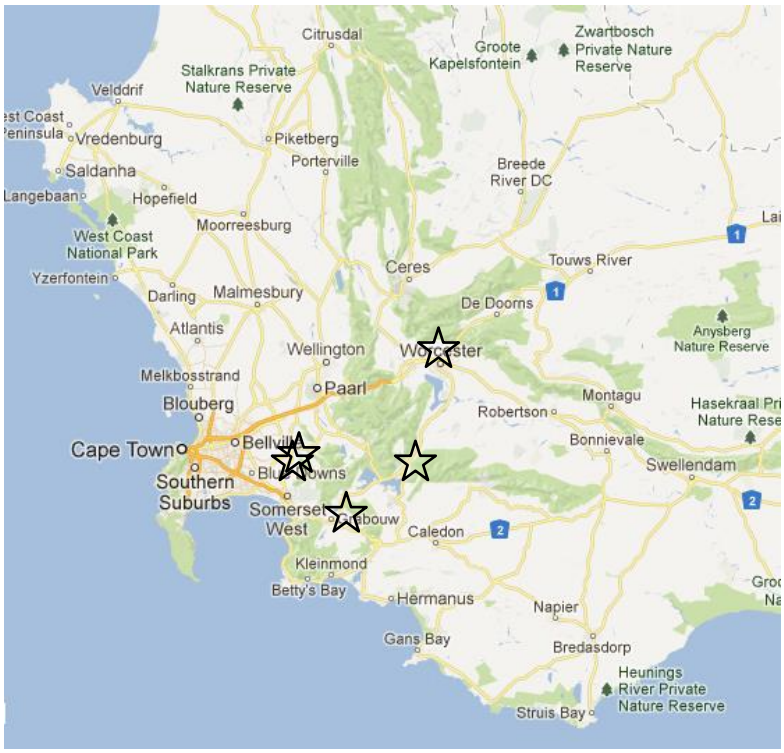

Figure 2.1: The location of five sampling sites used for tephritid trapping and fruit damage assessments from February 2013 to August 2014 in the Western Cape Province, South Africa. The sites are indicated as empty stars.

Fruit fly trapping

Five Multi-lure traps (Better Trap Manufacturing Inc, Fresno, CA, USA), each containing a different lure and a 1×1.5cm strip of killing agent, DDVP (2,2-Dichlorovinyl dimethyl phosphate, Chempac (Pty), Ltd, Paarl, South Africa), were hung in or near known host trees at a height between 1.5 and 2m. Table 2.1 gives a summary of the host plants in which traps were hung at each of the sampling sites.

The five lures used were BioLure® (Chempac (Pty) Ltd, Paarl), cuelure (Chempac (Pty) Ltd, Paarl), methyl eugenol (Chempac (Pty) Ltd, Paarl), PheroLure™ (Insect Science™, Tzaneen, Limpopo Province, South Africa) (an enriched ginger root oil (EGO)) and terpinyl acetate (TA). These were selected as they attract a wide range of species of fruit flies reported to occur in the Western Cape. Biolure, EGO and terpinyl acetate specifically attract *Ceratitis* spp., while cuelure and methyl eugenol attract species within the genera *Dacus* and *Bactrocera* (Manrakhan & Addison, 2007). The lures and killing agent were replaced every four weeks throughout the experiment. The field lifetime of methyl eugenol (ME) and cuelure (CUE) has been recorded as 6 weeks (IAEA, 2003). BioLure® (BIO) is a synthetic female attractant that consists of three components, namely ammonium acetate, trimethylamine hydrochloride and putrescine (Leza et al., 2008) and has been reported to trap *C. capitata* and *C. rosa*. Lures aimed at attracting females are based on food or host odours (IAEA, 2003). CUE and ME (4-allyl-1,2-dimethoxybenzene-carboxylate) are parapheromones (male-specific) (Vayssières et al., 2009), which are highly attractive to a number of *Bactrocera* and *Dacus* species (IAEA, 2003) They were included in the study as part of an early detection system to determine the presence of invasive fruit flies in the Western Cape. TA has been reported to attract *C. rosa* (White and Elson-Harris, 1992) and EGO lure has been recommended for the monitoring and trapping of *Ceratitis* species (Shelly & Pahio, 2002; Mwatawala et al., 2012).

Table 2.1: Host trees in which Multi-lure traps, baited with different lures, were hung during field sampling of two *Ceratitis* fruit fly species from February 2013 to August 2014, in the Western Cape, South Africa.

SITE	LURE*	HOST TREE
Stellenbosch botanical gardens	BIO	*Mangifera indica* L.
	CUE	*Mangifera indica* L.
	EGO	*Psidium guajava* L.
	ME	*Syzigium jambos* L.
	TA	*Musa sapientum* Kuntze
Stellenbosch, Welgeleë lodge	BIO	*Psidium guajava* L.
	CUE	*Cydonia oblonga* Mill.
	EGO	*Prunus japonica* Thunb.
	ME	*Vitis vinifera* L.
	TA	*Citrus limon* Osbeck
Elgin, Bon acres	BIO	*Eriobotrya japonica* Lindl.
	CUE	*Ficus carica* L.
	EGO	*Morus nigra* L.
	ME	*Vitis vinifera* L.
	TA	*Prunus persica* Sieb. & Zucc.
Villiersdorp, Home garden	BIO	*Pyrus communis* L.
	CUE	*Prunus persica* Sieb. & Zucc.
	EGO	*Psidium guajava* L.
	ME	*Ficus carica* L.
	TA	*Prunus cerasifera* Ehrh.
Worcester, Summerhill guest farm	BIO	*Musa sapientum* Kuntze
	CUE	*Prunus japonica* Thunb.
	EGO	*Ficus carica* L.
	ME	*Ficus carica* L.
	TA	*Prunus persica* Sieb. & Zucc.

*BIO = Biolure; EGO = Pherolure; CUE = Curelure; ME = Methyl eugenol; TA = Terpinyl acetate.

The traps were serviced every 2 weeks during spring and summer months and once every 4 weeks during autumn and winter months.

Servicing traps consisted of brushing out any tephritids into specimen jars and marking the jars with the date, location, and the lure used for each specific trap. These jars were then taken back to the laboratory where the flies were identified, counted and sexed in order to establish the relative distribution and abundance of the fruit flies for specific times of the year. Males were distinguished between sterile and wild males through the use of an ultra violet light source in a dark room. Sterile flies were marked with fluorescent dye, and were removed so that only the wild population of fruit flies were used during statistical analyses.

Fruit damage assessment

Along with servicing the traps, fruits were collected at the five sites, depending on fruit availability. The majority of fruits that were collected had fallen from the host plant and only a few fruits were ever picked from the host plant itself. Fruit collections from the host plant at the sites were capped by the home owners as these fruits are harvested for personal use. The collected fruits were taken back to the laboratory and were weighed before being placed on a 2 - 3cm layer of sterilized sand (Malmesbury) in plastic containers (19×19×18cm). In this study, collected fruits were only weighed and not counted as some of the collected fruits were already reduced to a pulp and were not distinct individual fruit units. The sand was sterilized by placing it in a -15°C freezer for 48 hours. A large square opening was cut from each of the lids of the plastic containers and resealed by gluing grey organza on the inside of the lids. The material allows for light and air movement into the container and is fine enough to prevent flies from escaping. These fruits were checked on a daily basis for adult fruit flies that had emerged and were kept until the fruits had decomposed to the point where they were dried

out and hard. Before discarding any dried fruit, the fruit was carefully opened for a final inspection of any larval or pupal presence. Natural host preference and the degree of fruit infestation could then be calculated for specific times of the year.

Vargas et al. (1983) found distinct patterns in the distribution of *C. capitata* in Hawaii through trap and fruit damage assessment data, and for this reason these methods were used in this study.

Statistical analysis

Due to the nature of the trapping method used in the present study, where only one trap of each lure was installed at each sampling location which was serviced once for every time interval, there was essentially only one replication for each trap. Since the specific aims of this study were to establish the population dynamics of *C. capitata* and *C. rosa* in home gardens and to determine the influence of lures in monitoring of the two fruit fly pest species, the different sampling sites were considered as different replicates. ME and CUE were excluded from further statistical analyses due to a high occurrence of zero values which affected the normality of the data and in turn the accuracy of the results. Data were log transformed to achieve normality and analysed using a linear regression model to determine the general effects of lure, species and sex, and mixed models to determine the effects of lure and time for each species and sex group. From here on, the term “all lures” or “all traps” refers to BIO, EGO and TA, unless otherwise stated. Trap data were standardized by using fruit flies per trap per day (FTD = F ÷ T × D) which was calculated by dividing the number of flies (F) collected for each trap by the number of days since the last service (D) (IAEA, 2003). The total number of traps serviced per sampling site (T) for each lure was equal to 1, thus FTD was calculated as FTD = F ÷ D.

Results and discussion

Fruit fly trapping

During the study period of 2013 and 2014, the total number of *C. capitata* and *C. rosa* specimens collected in Multi-Lure traps from the five sampling sites amounted to 39 285. Of the 39 285 flies trapped, 4248 were sterile *C. capitata* males leaving a total of 35 037 flies that represent the wild populations of these two species of fruit flies. Only the wild populations of fruit flies were used for statistical analyses. The following percentages, in descending order, of 73.11%, 19.69%, 6.91%, 0.26% and 0.03% were caught in traps baited with EGO, BIO, TA, CUE and ME respectively. Looking at the total number of flies trapped at the different sampling areas, 44.95%, 34.45%, 13.80% and 6.80% were trapped in Worcester, Villiersdorp, Stellenbosch and Elgin, respectively. The number of FTD at each of the locations in descending order for *C. capitata* was Worcester (males = 606.99, females = 57.43), Villiersdorp (males = 514.20, females = 66.18), Elgin (males = 108.48, females = 2.85) and Stellenbosch (males = 83.85, females = 21.09). The number of FTD at each of the locations in descending order for *C. rosa* was Stellenbosch (males = 34.38, females = 43.69), Elgin (males = 18.11, females = 1.11), Villiersdorp (males = 11.70, females = 0.40) and Worcester (males = 4.24, females = 0.13). Tephritid by-catch is summarized in Table 2.2 below. As numbers caught were low and intermittent, the data were not subjected to any statistical analyses.

Table 2.2: Non-target tephritid species caught in traps from February 2013 to August 2014. The number caught is indicated in brackets following the species name.

LURE	SPECIES	LOCATION	DATE
EGO	*Ceratitis munroanum* (1)	Stellenbosch	07/11/13
	Ceratitis munroanum (1)	Villiersdorp	21/11/13
	Ceratitis munroanum (1)	Villiersdorp	19/12/13
	Ceratitis munroanum (1)	Villiersdorp	16/01/14
	Dacus ciliatus Loew. (1)	Stellenbosch	13/03/14
	Dacus ciliatus Loew. (1)	Stellenbosch	17/04/14

Cont.

	Dacus ciliatus Loew. (1)	Stellenbosch	02/05/14
	Dacus ciliatus Loew. (1)	Villiersdorp	02/05/14
	Dacus ciliatus Loew. (1)	Villiersdorp	02/05/14
	Dacus ciliatus Loew. (1)	Worcester	13/02/14
	Ceratitis spp. (1)	Villiersdorp	16/01/14
	Tephritid sp. 1 (1)	Stellenbosch	02/05/14
	Tephritid sp. 2 (2)	Stellenbosch	02/05/14
BIO	*Dacus ciliatus* Loew. (1)	Villiersdorp	27/02/14
	Dacus ciliatus Loew. (2)	Villiersdorp	13/03/14
	Dacus ciliatus Loew. (1)	Worcester	21/11/13
	Dacus ciliatus Loew. (1)	Worcester	19/12/13
	Dacus ciliatus Loew. (2)	Worcester	17/04/14
	Dacus ciliatus Loew. (4)	Worcester	16/05/14
	Dacus ciliatus Loew. (4)	Worcester	16/05/14
	Dacus spp. (1)	Worcester	16/05/14
ME	Tephritid sp. 3 (1)	Villiersdorp	05/12/13
CUE	*Dacus vertebratus* Bezzi (1)	Worcester	17/04/14
TA	*Dacus ciliatus* Loew. (1)	Worcester	16/01/14
	Dacus ciliatus Loew. (1)	Worcester	13/02/14

In general, the number of FTD for *C. capitata* was significantly higher ($F_{(1,1139)} = 81.28$, $p < 0.001$) than *C. rosa*. There were significantly ($F_{(1, 1139)} = 71.85$, $p < 0.001$) more males collected for both species of fruit flies. The number of FTD for *C. capitata* and *C. rosa* was significantly different per month ($F_{(22, 1139)} = 5.83$, $p < 0.001$) (Fig. 2.2).

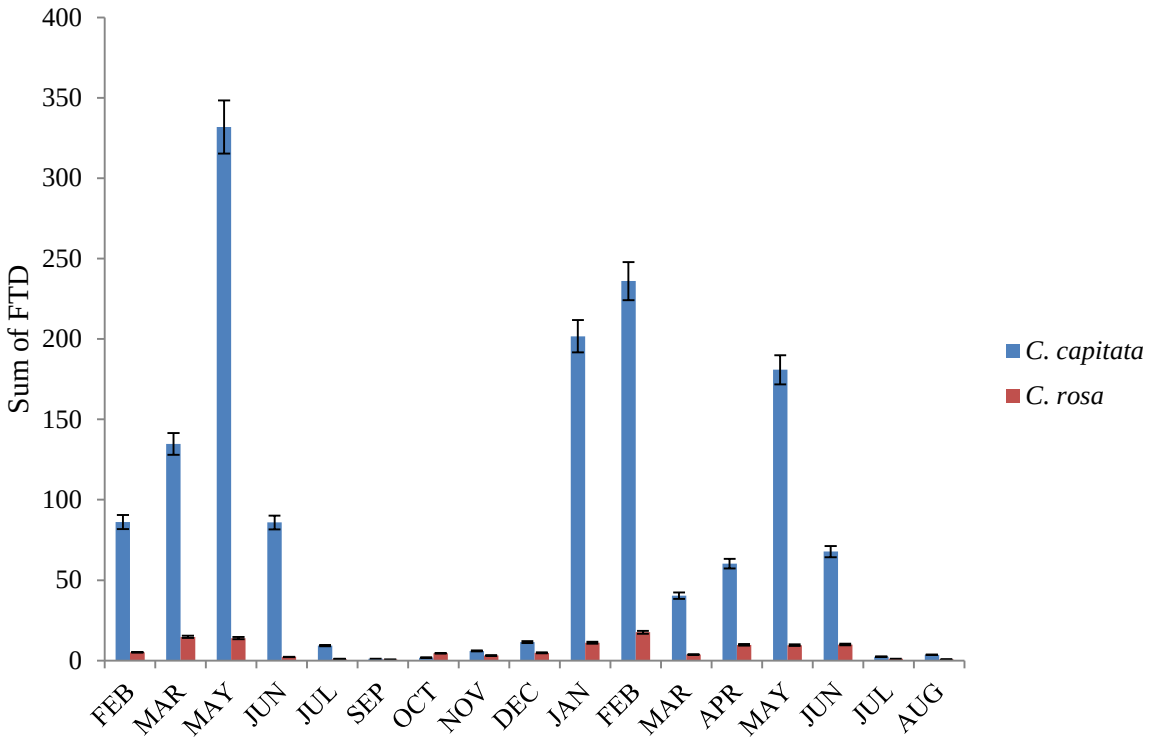

Figure 2.2: The sum of fruit flies per trap per day (FTD) for *Ceratitis capitata* and *C. rosa* males and females grouped over five different sampling sites and lures in the Western Cape from February 2013 to August 2014. Error bars denote 95% confidence intervals.

The abundance of *C. capitata* males and females, and *C. rosa* males, in terms of FTD were significantly influenced by the month of the year in which they were caught. The type of lure used in the traps had a significant effect on the number of flies caught from both species and both sexes. An interaction was found between the month of the year and the different lures that were used in each of the traps, this interaction significantly influenced the number of female *C. capitata* FTD only (Table 2.3).

Table 2.3: Parameters of mixed model analysis to determine the effects of month and lure, and interactions between month and lure on catches of males and females of *Ceratitis capitata* and *C. rosa* sampled from February 2013 to August 2014 in the Western Cape.

Species	Effects	df	F	p
C. capitata				
Female	Month	22	2.243	0.002
	Lure	2	37.246	< 0.001
	Month*Lure	44	1.756	0.005
Male	Month	22	5.265	< 0.001
	Lure	2	57.856	< 0.001
	Month*Lure	44	1.326	0.098
C. rosa				
Female	Month	22	0.469	0.981
	Lure	2	16.547	< 0.001
	Month*Lure	44	0.467	0.998
Male	Month	22	2.461	0.001
	Lure	2	39.877	< 0.001
	Month*Lure	44	0.908	0.639

Ceratitis capitata was observed to be the most abundant in March and May 2013, and January, February and May 2014, with relatively low numbers from July to December (Fig. 2.2 and Table 2.4). *Ceratitis rosa* also had the highest abundance during March and May 2013, and January, February, April, May and June 2014. The numbers of *C. rosa* were also relatively low from July to December (Fig. 2.2 and Table 2.4). These findings are in line with those of Myburgh (1956), who observed that the highest abundances for these two fruit fly species in the Western Cape were during March, April and May, and the months of lowest abundances were in June, July and August. Nyamukondiwa et al. (2013) reported that insects typically occupy thermal niches which are ideal for their short-term performance as well as their long-term survival. In these niches, during winter periods, low temperatures can influence the survival of these insects either directly (freezing) or indirectly (delayed development) which changes the population dynamics (Nyamukondiwa et al., 2013).

Table 2.4: The sum of *Ceratitis* fruit flies per trap per day (FTD) with respect to species, sex, lure and the month of the year, sampled from February 2013 to August 2014. Colours represent abundance of flies on a continuum, with dark green representing low FTD and red representing high FTD.

	FEB	MAR	MAY	JUN	JUL	SEP	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG
C. capitata	**86.14**	**134.71**	**331.81**	**85.89**	**9.31**	**1.09**	**1.74**	**5.93**	**11.50**	**201.71**	**236.00**	**40.29**	**60.29**	**180.90**	**67.73**	**2.36**	**3.61**
Female	**11.43**	**25.93**	**30.56**	**8.11**	**0.93**	**0.11**	**0.15**	**0.21**	**1.50**	**19.71**	**19.79**	**2.86**	**8.00**	**15.59**	**2.29**	**0.18**	**0.21**
BIO	11.36	25.79	28.70	7.54	0.86	0.08	0.00	0.14	0.71	18.11	16.64	2.64	7.86	14.79	2.20	0.11	0.21
CUE	0.00	0.00	0.18	0.18	0.05	0.00	0.00	0.00	0.57	0.04	0.00	0.07	0.00	0.41	0.07	0.04	0.00
EGO	0.07	0.07	0.13	0.04	0.00	0.03	0.07	0.07	0.00	0.89	2.57	0.14	0.03	0.03	0.00	0.00	0.00
ME	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00
TA	0.00	0.07	1.54	0.36	0.00	0.00	0.08	0.00	0.21	0.68	0.57	0.00	0.11	0.34	0.02	0.00	0.00
Male	**74.71**	**108.79**	**301.24**	**77.79**	**8.38**	**0.98**	**1.59**	**5.71**	**10.00**	**182.00**	**216.21**	**37.43**	**52.29**	**165.31**	**65.44**	**2.18**	**3.39**
BIO	6.21	22.21	30.45	10.32	0.64	0.08	0.00	0.00	0.07	6.29	7.57	2.29	4.03	9.76	2.78	0.11	0.18
CUE	0.00	0.07	1.35	0.14	0.02	0.00	0.00	0.00	0.00	0.04	0.21	0.21	0.11	0.14	0.15	0.04	0.00
EGO	66.00	86.07	214.30	59.32	6.76	0.44	1.52	5.64	9.29	164.43	204.29	34.79	46.60	135.21	59.73	1.93	3.04
ME	0.21	0.14	0.02	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00
TA	2.29	0.29	55.13	8.00	0.95	0.47	0.07	0.00	0.64	11.25	4.14	0.14	1.51	20.21	2.78	0.11	0.18
C. rosa	**5.07**	**14.71**	**13.95**	**2.29**	**1.00**	**0.77**	**4.55**	**3.14**	**4.79**	**11.14**	**17.64**	**3.79**	**9.71**	**9.55**	**9.90**	**1.11**	**0.64**
Female	**3.00**	**11.14**	**6.93**	**0.46**	**0.31**	**0.22**	**0.07**	**0.57**	**1.71**	**4.14**	**3.50**	**1.36**	**4.00**	**4.31**	**3.02**	**0.29**	**0.29**
BIO	3.00	11.14	6.91	0.46	0.29	0.20	0.07	0.57	1.64	4.04	3.43	1.36	4.00	4.24	3.02	0.29	0.25
CUE	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.00
EGO	0.00	0.00	0.02	0.00	0.02	0.02	0.00	0.00	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ME	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
TA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.07	0.00	0.00	0.00	0.00	0.00	0.04
Male	**2.07**	**3.57**	**7.02**	**1.82**	**0.69**	**0.55**	**4.48**	**2.57**	**3.07**	**7.00**	**14.14**	**2.43**	**5.71**	**5.24**	**6.88**	**0.82**	**0.36**
BIO	0.29	2.29	1.35	0.04	0.10	0.05	0.29	0.36	0.43	1.36	2.43	0.29	0.49	1.07	2.44	0.07	0.07
CUE	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.03	0.00	0.00	0.00
EGO	1.50	1.29	5.22	1.64	0.55	0.38	3.58	2.14	2.36	4.43	10.86	2.00	4.54	3.48	3.80	0.39	0.07
ME	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
TA	0.29	0.00	0.45	0.14	0.02	0.13	0.60	0.07	0.29	1.18	0.86	0.14	0.69	0.66	0.63	0.36	0.21
Grand Total	**91.21**	**149.43**	**345.76**	**88.18**	**10.31**	**1.86**	**6.29**	**9.07**	**16.29**	**212.86**	**253.64**	**44.07**	**70.00**	**190.45**	**77.63**	**3.46**	**4.25**

Table 2.5: Number of *Ceratitis* fruit flies per trap per day (FTD) for five different lures, with respect to fruit fly species and sex, sampled from February 2013 to August 2014.

SPECIES	**LURE***				
	BIO	**CUE**	**EGO**	**ME**	**TA**
C. capitata	**240.79**	**4.09**	**1103.50**	**0.54**	**111.99**
Female	137.73	1.61	4.16	0.06	3.99
Male	103.06	2.48	1099.34	0.48	108.00
C. rosa	**58.29**	**0.16**	**48.37**	**0.00**	**6.92**
Female	44.91	0.07	0.13	0.00	0.21
Male	13.38	0.09	48.24	0.00	6.71

*Lures: BIO = Biolure; CUE = Cuelure; EGO = Pherolure; ME = Methyl eugenol; TA = Terpinyl acetate

The number of FTD for *C. capitata* was higher than *C. rosa* for each of the lures used (Table 2.5). Manrakhan & Addison (2007) found that *C. rosa* was only present in some parts of the Western Cape, with a lower abundance in traps and infested fruits compared to *C. capitata*. EGO and TA attracted more males than females for both species of fruit flies (Table 2.5). Mwatawala et al. (2012) caught significantly higher numbers of *Ceratitis* males (96% of N = 3824) in EGO traps compared to trimedlure. BIO attracted more females of both species (Fig. 2.3). Manrakhan & Kotze (2011) found that more *C. capitata*, *C. rosa* and *C. cosyra* females were attracted to protein hydrolysate baits than males of the tested species. Kaspi et al. (2002) demonstrated that females that were exposed to a protein diet increased development in terms of developmental time, size and reproductive success. Males of *C. capitata* and *C. rosa* were also attracted to some extent by BIO. Males also experience certain developmental benefits when feeding on a food containing protein, such as shorter developmental times and sexual behavioural advantages such as an increased likeliness for participation in leks (Kaspi et al., 2002) and this probably explains their attraction to the food-based attractant BIO.

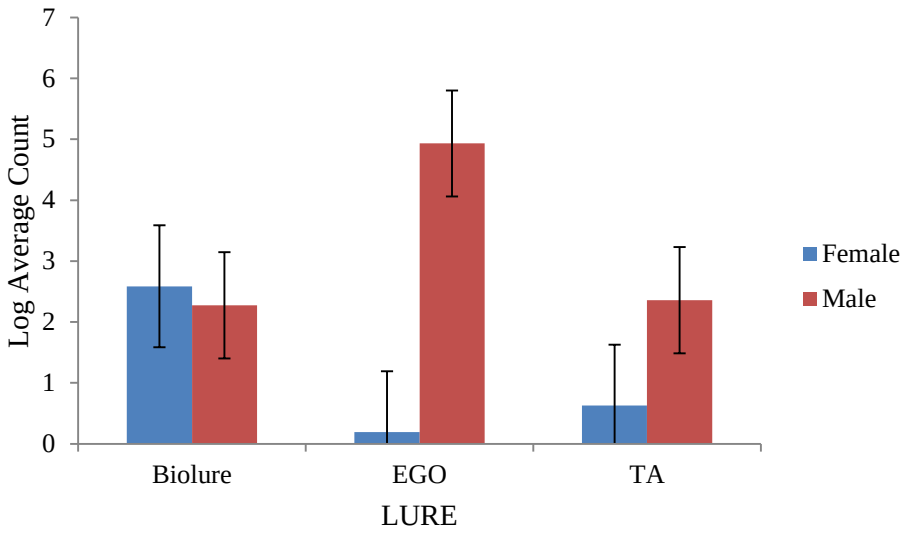

Figure 2.3: The log values of the average number of male and female *Ceratitis capitata* and *C. rosa* trapped during a field assessment from February 2013 to August 2014 in the Western Cape, South Africa. Error bars denote 95% confidence intervals.

Fruit damage assessment

Assessment of damage on fruit in an area is important in order to establish the breeding sites of flies within the specific area. Table 2.6 summarizes the number of adult fruit flies that successfully emerged from the different fruits and vegetables collected in home gardens. Only hosts from which adults successfully emerged are presented in Table 2.6. These hosts hold high potential as alternate hosts of *C. capitata* and *C. rosa*. Where a host was infested by only one of the species of fruit fly, it does not necessarily indicate that it is not a potential host for the other species (Myburgh, 1956). The infestation index used (adults/kg fruit) enables a comparison between different fruit species as potential hosts for fruit flies irrespective of the number of fruits sampled and the size of the individual fruits (Mwatawala et al, 2009).

Table 2.6: Adult emergence per kg fruit for *Ceratitis capitata* and *C. rosa* from host plants collected on the ground in home gardens at five sampling sites in the Western Cape from February 2013 to August 2014.

FRUIT	WEIGHT (g)	CAP M	CAP F	ROSA M	ROSA F	CAP M/kg FRUIT	CAP F/kg FRUIT	ROSA M/kg FRUIT	ROSA F/kg FRUIT	CAP/kg FRUIT	ROSA/kg FRUIT
Apples*	4837.63	22	28	1	6	4.548	5.788	0.207	1.240	10.336	1.447
Bell peppers**	373.65	4	3	0	0	10.705	8.029	0.000	0.000	**18.734**	0.000
Figs**	1176.25	6	6	0	0	5.101	5.101	0.000	0.000	**10.202**	0.000
Grapes*	331.40	0	2	0	0	0.000	6.035	0.000	0.000	**6.035**	0.000
Guavas**	2573.31	47	51	145	128	18.264	19.819	56.348	49.741	**38.083**	**106.089**
Jambos**	442.00	0	0	65	59	0.000	0.000	147.059	133.484	0.000	**280.543**
Kei-apples**	2999.31	1	4	5	2	0.333	1.334	1.667	0.667	**1.667**	**2.334**
Lemons**	1982.24	1	1	0	0	0.504	0.504	0.000	0.000	**1.008**	0.000
Peaches*	1389.97	37	39	2	2	26.619	28.058	1.439	1.439	54.677	2.878
Pears*	5271.41	138	143	8	9	26.179	27.127	1.518	1.707	53.306	3.225
Piquanté peppers**	578.58	15	18	0	0	25.926	31.111	0.000	0.000	**57.036**	0.000
Plums*	574.46	2	1	0	0	3.482	1.741	0.000	0.000	5.223	0.000

* Preferred hosts, ** Alternative hosts, Infestation indices of alternate hosts are highlighted in red.

Almonds, clementines, custard apples, Eugenia berries, gooseberries, granadillas, Kaffir plums, mangoes, mulberries, nectarines, olives, pomegranates, potatoes, quinces, red chilli peppers, strawberry tree fruits and tomatoes were also collected but returned no emergence of either species of fruit flies. It is recommended that all fleshy fruit or vegetables should still be included in future damage assessments, as various factors could have played a role in not finding any emergence from these fruits and vegetables in the present study.

José et al. (2013) found that *Bactrocera dorsalis* (previously *B. invadens*) was the most abundant species during a study in Northern Mozambique, reaching an infestation index of 141.6 adults/kg on guava. During a study on *B. dorsalis* in Kenya, Rwomushana et al. (2008) reported that a high infestation index can be considered to be above 100 adults/kg of host plant. Compared to the infestation indices in these studies, the infestation index of 106.089 adults/kg guava and 280.543 adults/kg jambos observed in the present study were very high (Table 2.6). Mwatawala et al. (2006) reported the highest percentage of fruit infestation by *B. dorsalis* in Tanzania on mangoes (61.7%) followed by guavas (37.5%), and Rwomushana et al. (2008) found fruit infestation of 32.9% on guavas by *B. dorsalis*. Myburgh (1956) reported guavas as an important alternate host for *C. capitata* and *C. rosa* and described the fruit as an excellent medium for larval development on which he observed considerably more rapid development of the larvae compared to that of apples and pears under comparable conditions (Myburgh, 1956). Guava should thus be considered as an alternate host with high potential for enabling persistence of *C. capitata* and *C. rosa* during non-fruiting seasons of commercial hosts in the Western Cape. It is important to consider the total biomass of a preferred host that is produced in a certain area for its importance in contributing to fruit fly abundance, and not just the degree to which single fruits from preferred hosts are infested (Vargas et al., 1983). Mwatawala et al. (2009) found that *C. rosa* frequently infests apples, pears and peaches. In the present study, infestation rates on these three fruits were found to be

higher for *C. capitata*. This could be due to the fact that *C. capitata* is still the predominant fruit fly species in the Western Cape and that *C. rosa* populations are relatively smaller.

Comparing the number of adult flies that emerge per kg of fruit from different areas may serve as an indication of the number of breeding flies in that area (Vargas et al., 1983). Standardizing a method for damage assessment studies would prove useful in building a robust database of different species of pests and could then be used to track relative abundance and distribution as well as host preference and alternate hosts of specific species.

The effect of host availability

Carey (1984) described that the presence of *C. capitata* is dependent on the availability of suitable hosts for the development of the immature stages and attributed abundance of these flies mainly to the presence of a few key hosts.

Table 2.7: The fruiting times of preferred (*) and alternate (**) hosts for *C. capitata* and *C. rosa* in the Western Cape, from which positive rearing was obtained.

HOST PLANT	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
Apple*	X	X	X	X								
Peach*	X	X								X	X	X
Pear*	X	X	X									X
Plum*	X	X	X								X	X
Grape*	X	X	X	X	X						X	X
Bell pepper**		X	X	X	X							
Fig**	X	X	X	X	X						X	
Guavas**				X	X							
Jambo**		X	X									
Kei-apple**		X	X		X							
Lemon**		X	X	X	X							
Piquanté peppers**		X	X	X	X							

The phenology of key or preferred hosts that are grown in the area surrounding sample sites, followed by alternate hosts that occurred at the sampling sites are summarized in Table 2.7.

For the alternate hosts in Table 2.7, only plants that were infested by either *C. capitata* or *C. rosa* were summarized. The phenologies for preferred hosts were obtained from Manrakhan & Addison (2014) and the phenology of alternate hosts was derived from the present study. Due to the fruiting times of alternate hosts that were only found during the same time as the preferred hosts in home gardens, and the scarceness of literature describing the fruiting phenologies of the alternate hosts used in this study, more sampling of any available host fruit between June and October is required in future studies. There is a possibility that flies overwinter as adults with no ovarian development at the low temperatures (Duyck & Quilici, 2002).

Referring to Table 2.4, Table 2.7 and Fig. 2.2, a trend can be seen where the abundances and distribution of the fruit flies, at certain times of the year, are primarily dominated by the availability of preferred (commercial) hosts. Studies have described that the mass movement of *C. capitata* is associated with the presence of host species (Christenson & Foote, 1960) and that the availability of suitable hosts drive fluctuating population numbers of certain tephritids throughout a year cycle (Mwatawala et al., 2009; Vayssières et al., 2009). Alternate host fruits are still important as they allow fruit flies to breed throughout the year until the preferred hosts become available again.

The effect of rainfall

Previous studies have shown that significant correlations between the total monthly rainfall and the abundances of tephritid fruit flies exist (Vargas et al., 1983; Vayssières et al., 2009; De Villiers et al., 2013). More so, studies have shown that the abundance of *C. capitata* is influenced by rainfall (Harris & Lee, 1989) and that *C. rosa* is more tolerant to wet conditions compared to *C. capitata* (De Villiers et al., 2013).

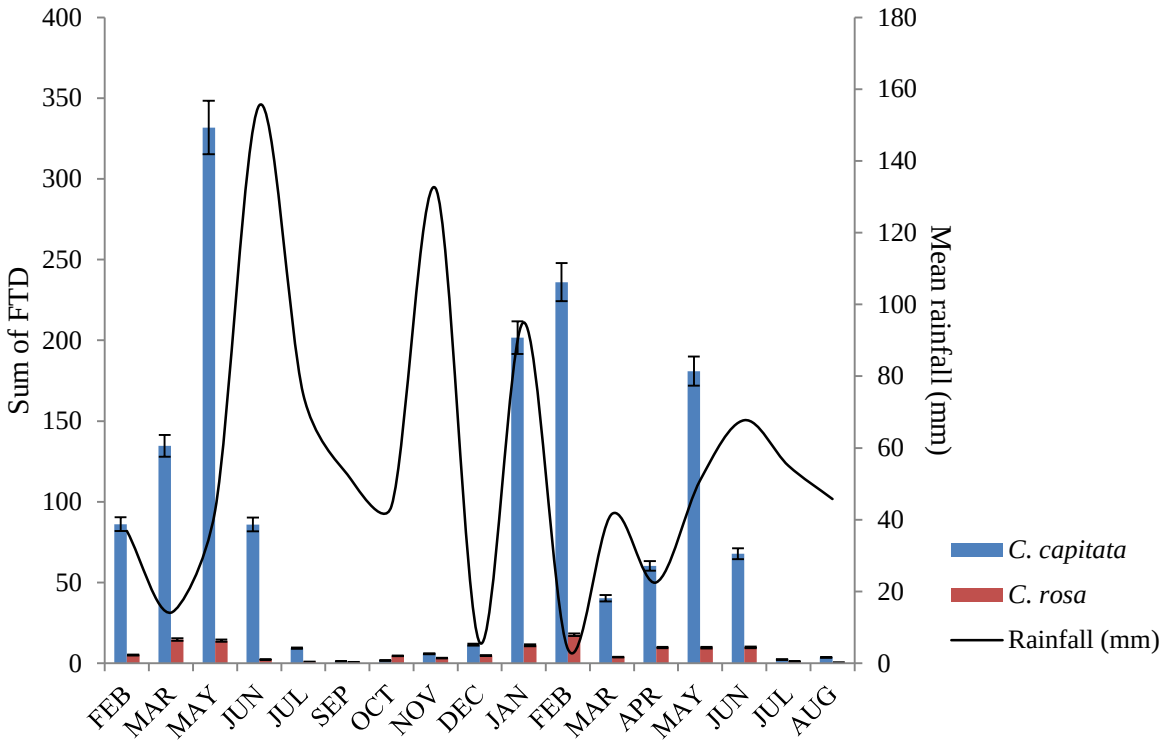

Figure 2.4: The sum of fruit flies per trap per day for *Ceratitis capitata* and *C. rosa* males and females grouped over five different sampling sites and lures in the Western Cape including the mean monthly rainfall from February 2013 to August 2014. Error bars denote 95% confidence intervals.

In the present study the highest abundance for both species of fruit flies occurred during the dry seasons and the lowest abundance during the wet seasons (Fig. 2.4). This concurs with findings of Vargas et al. (1983) where the highest trap catches occurred in dry seasons compared to low trap catches during wet seasons and is possibly due to the fact that the Western Cape is located in a winter rainfall area where periods of high rainfall are associated with low temperatures.

The effect of temperature

Two of the most important environmental factors that determine population dynamics of any insect population, are water availability and temperature which affects biochemical and physiological processes of insects (Nyamukondiwa & Terblanche, 2009). In Fig. 2.5,

population numbers of both *C. capitata* and *C. rosa* peak during months where mean temperatures are more than 20°C. Nyamukondiwa & Terblanche (2009) found that the critical minimum temperatures for *C. capitata* and *C. rosa* were similar (5.4 – 6.6°C), but the critical maximum temperature for *C. capitata* (42.4 – 43.0 °C) was significantly higher than *C. rosa* (41.8 – 42.4°C). Nyamukondiwa et al. (2013), in a similar study on the thermal biology of *C. capitata* and *C. rosa*, found that the lower temperatures at which 90% mortality occurred during an 8h exposure, for larvae were -3.01°C (*C. capitata*) and -2.93°C (*C. rosa*), for pupae, -6.25°C (*C. capitata*) and -4.91°C (*C. rosa*), and for adults -2.58°C (*C. capitata*) and -3.01°C (*C. rosa*). Nyamukondiwa et al. (2013) found the upper lethal temperature where 50% mortality occurred, over a range of different exposure times, was the same for *C. capitata* (38.02°C) and *C. rosa* (37.85°C). An explanation for the population numbers of *C. capitata* and *C. rosa* that were lower during colder periods could be due to physiological processes not occurring optimally which drives a species to either migrate, hibernate or die (Cossins & Bowler, 1987; Chown & Nicolson, 2004). Vera et al. (2002) when predicting potential distribution of *C. capitata* in Argentina and Australia by using meteorological data, identified temperature as being a major determining factor of the distribution of this species.

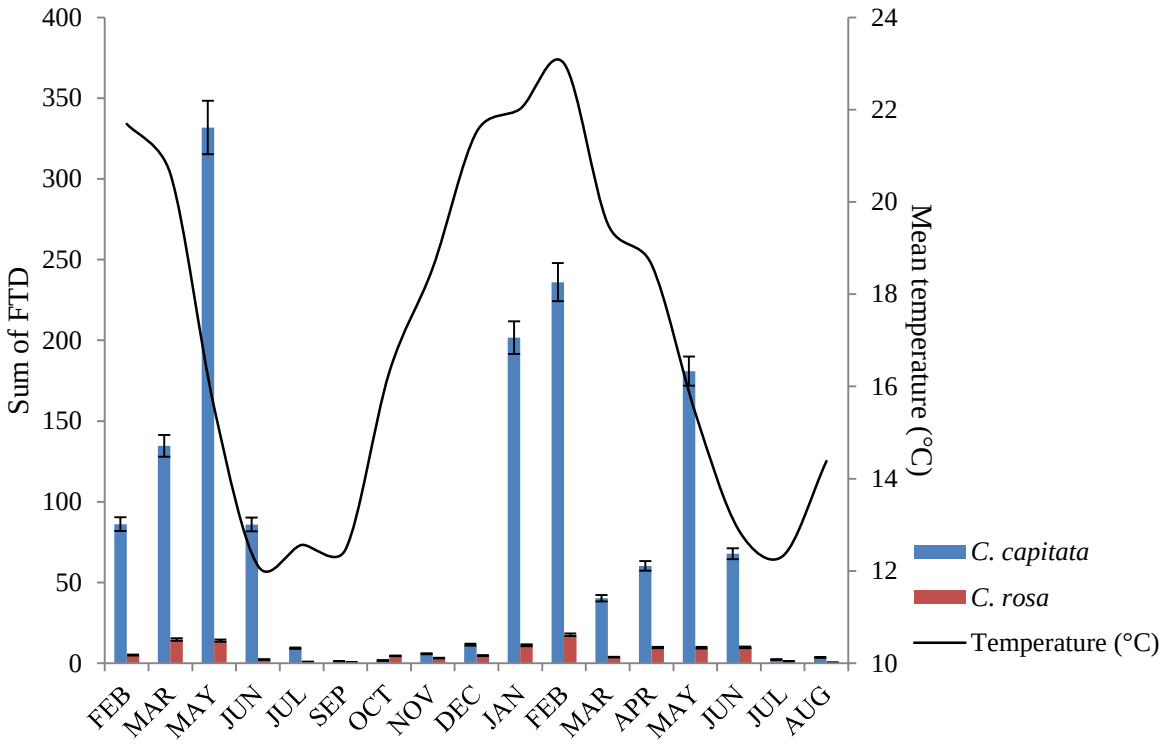

Figure 2.5: The sum of fruit flies per trap per day for *Ceratitis capitata* and *C. rosa* males and females grouped over five different sampling sites and lures in the Western Cape including the mean monthly temperatures from February 2013 to August 2014. Error bars denote 95% confidence intervals.

Since temperatures in the Western Cape rarely rise above 35°C, *C. capitata* and *C. rosa* are able to distribute and survive throughout the Province during the warmer summer months. It is the colder temperatures throughout winter that are likely to suppress the population numbers, but all life stages of both species of fruit fly should withstand winters in the Western Cape which rarely dips below 0°C (Nyamukondiwa et al., 2013). Temperature alone could not be used to explain the fluctuation in population numbers of the two species of fruit flies.

The effect of elevation

During previous studies, correlations between the altitude of sampling sites and the abundance of fruit flies have been observed. In the present study, a pattern was observed where *C. capitata* abundance was higher at higher altitudes and the inverse was found for *C.*

rosa (Fig. 2.6). Vargas et al. (1983) reported *C. capitata* to have higher abundance at lower elevations < 300m and lower abundance at high elevations > 600m. The results of the present study are different from Mwatawala et al. (2009) who reported that *C. rosa* was more prevalent in coffee plantations at higher altitudes of 1650m. In a study by Ekesi et al. (2009), levels of mango infestation by *B. dorsalis* were found to be indirectly proportional to the elevation of sampling sites and that *B. dorsalis* displaced *C. cosyra* in highland areas. The same pattern has been reported in Hawaii where *B. dorsalis* displaced *C. capitata* at higher altitudes (Vargas et al., 1995). Israely et al. (2005) trapped significantly more *C. capitata* flies per trap per week at higher altitudes of 600 - 900m compared to lower altitudes of 100 – 400m in Israel.

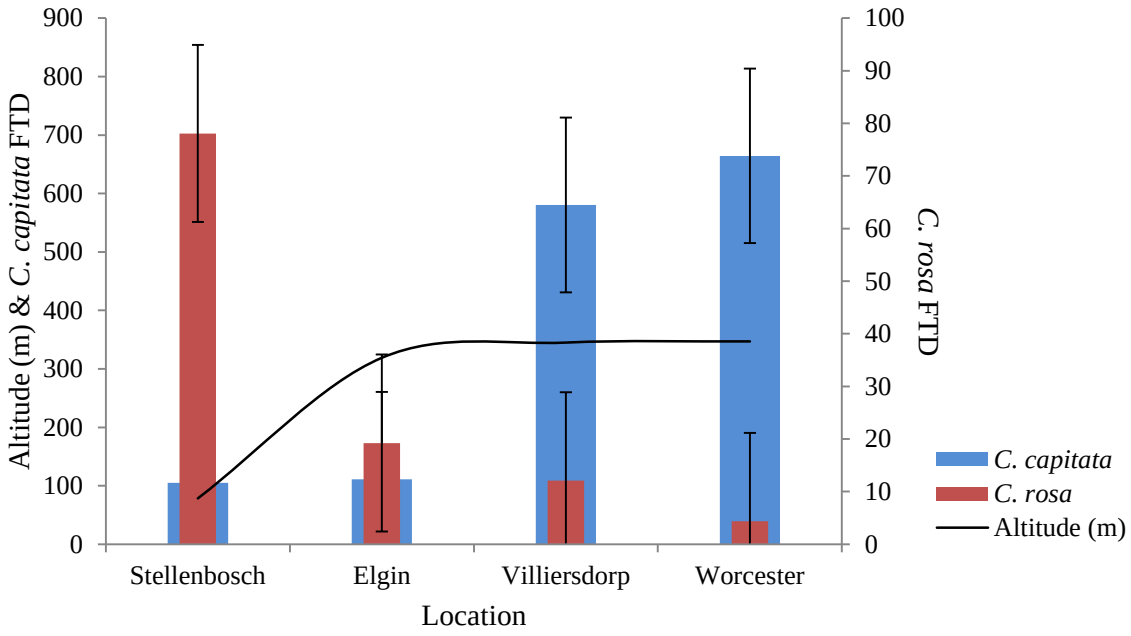

Figure 2.6: The sum of *Ceratitis* fruit flies collected per trap per day (FTD) from five different lure traps at five sampling locations against the altitude of these sampling locations from February 2013 to August 2014. The two years were grouped as the observed pattern was the same. Two sampling sites in Stellenbosch were combined and the average altitude has been used. Error bars denote 95% confidence intervals.

Conclusion

It is important to consider that a factor such as elevation or rainfall, by itself, can not be used to explain differences in spatial and temporal distribution and abundance of the fruit flies but that it is rather a combination of abiotic and biological factors working together such as elevation, annual rainfall, temperature, host availability, competing tephritids and parasites (Vargas et al., 1983; Vayssières et al., 2009; Nyamukondiwa & Terblanche, 2009; De Villiers et al., 2013). Results in the present study indicate that the distribution and population dynamics of the two fruit fly pest species are the results of an intricate interaction between the effects of abiotic and biotic factors since none of the factors on their own could reliably explain the observed distribution in space and time. Vera et al. (2002) quantified climatic factors related to the distribution and abundance of *C. capitata* in the Mediterranean region and developed a model that, when applied to meteorological data, indicated potential distribution and abundance of the species in Australia and Argentina. Quantifying climatic factors related to the distribution of *C. capitata* and *C. rosa* in the Western Cape and developing a similar model using a software platform such as GIS in conjunction with meteorological data for Western Cape would prove a very useful tool in the management of these two species of fruit flies, and to supplement other biological data. Israely et al. (2005) suggest that effective management should be achieved through treatment campaigns in large areas, where area-wide management does not necessarily mean that the whole area needs to be treated, but rather that smaller areas within the large management area are treated according to knowledge of overwintering populations for the specific climatic and geographic locations. Israely et al. (2005) also focuses on a GIS-based approach for the management of *C. capitata*.

A list of alternate hosts for *C. capitata* and *C. rosa* needs to be established in order to improve the area-wide management of these species. Alternate hosts, especially in home

gardens near areas of commercial farming, provide a refuge for fruit flies until the next fruiting season of preferred hosts occur (Mwatawala et al., 2009; Manrakhan & Addison, 2014). In the present study all alternative hosts, sampled in home gardens, fall into the same fruiting time as the commercial hosts sampled. More sampling of the alternate hosts, on which infestation occurred in the present study, is needed to confirm whether they could serve as a refuge for the fruit flies during periods when preferred hosts are absent or not as abundant.

A good starting point for the management of these fruit flies is to manage the host plants with the highest infestation rates, with particular focus on guavas and jambos. This should be an on-going process and should preferably start prior to the implementation of an effective area-wide integrated pest management (AW-IPM) programme (Mwatawala et al., 2009). EGO and BIO is recommended for population surveys of the two species as these two lures trapped 74.63 and 18.17% of all flies in the present study, respectively. EGO mainly attracted males of *C. capitata* and *C. rosa* and BIO mainly attracted female flies of these two species. If screening surveys are done for detecting the presence of invasive fruit flies belonging to the genus *Bactrocera*, then lures such as ME and CUE are recommended.

Mwatawala et al. (2009) suggest that when alternate hosts that display high incidences of infestation occur in or near an area of commercial orchards, these plants should be removed. Some of these alternate hosts occur in home gardens and it is not always possible to remove the plant. For this reason it would be important to launch a survey on fruiting plants that occur in private home gardens near commercial farming areas in order to incorporate home gardens that hold high incidence of alternate hosts into the AW-IPM programme.

It is recommended for similar studies that more sampling sites be included in order to better reflect patterns of relative distribution and abundance of fruit fly pests and to prevent

vagueness of effects such as rainfall, temperature and elevation. At least five sampling sites should be used in each area studied, in order to statistically compare differences in the abundance and distribution between areas. In order to acquire optimal accuracy of data, such as monthly rainfall and mean monthly temperature, rain gauges and Thermochron® iButtons® (Maxim Integrated™) should be used at each of the sampling sites for the duration of the study period. Increasing the length of time spent on surveying will accordingly lead to more accurate findings and make such a study more robust and comprehensive.

In order to better manage fruit fly invasions, biological factors linked to the distribution of the fruit fly species should be controlled. It is evident that the *C. capitata* spread that occurred in many areas of Hawaii is strongly linked to human introduction of invasive fruiting trees and plants as well as the expansion of cultivation of indigenous fruit plants that became feral and widely distributed (Vargas et al., 1983). Karsten et al. (2013) suggest that human-mediated invasions are a main cause of fruit fly dispersal and gene flow among populations over long distances. Due to human-mediated mechanisms of dispersal, the connectivity of fruit fly populations in South Africa is too high for executing localized control programmes and that the movement of fruit between different regions of the country should be restricted until it is declared pest-free (Karsten et al., 2013).

As a result of an ever-increasing human population and its associated demand for food security, care should be taken when expanding different commercial fruit and vegetable growing practices, as this will mediate the expansion of the range and distribution of associated fruit fly species (Vargas et al., 1983).

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Chapter 3: The development of *Ceratitis capitata* (Wiedemann) and *C. rosa* Karsch (Diptera: Tephritidae) on different commercially grown hosts

Introduction

Ceratitis capitata and *C. rosa* are two highly polyphagous fruit fly species that are able to utilize more than 370 (Copeland et al., 2002) and 107 (De Meyer et al., 2002) species of host plants, respectively. *Ceratitis capitata* and *C. rosa* have been reported to co-infest certain fruits (Duyck et al., 2006) and differences in the relative abundance and distribution of these two species of fruit flies have been reported for the Western Cape Province (De Villiers et al., 2013). These differences may be explained by variability in the quality and nutritive values of different fruits and vegetables (Mayer, 1997), which potentially create a variety of nutritional options for fruit flies. Differences in quality and nutritive values even occur between individual plants of the same species depending on factors such as genetic variation and the microclimate in which they grow (Watson et al., 2002; Calenge et al., 2006). These variations in quality have also been reported to occur among fruits from the same tree and even in different areas inside an individual fruit due to the sugars, and other mineral contents, that increases or fluctuates at different rates as different areas of the fruit become ripe (Fernandes-da-Silva & Zucoloto, 1997).

Fruit flies are holometabolic pests which mean that the female, through the act of oviposition, decides the future host and diet of her offspring (Fernandes-da-Silva & Zucoloto, 1997), but it is still up to the larvae to locate the optimal area to feed inside the fruit in order to achieve their optimal development (Zucoloto, 1987; Zucoloto, 1991; Fernandes da-Silva & Zucoloto, 1993; Fernandes-da-Silva & Zucoloto, 1997; Canato & Zucoloto, 1998). The quality and nutritive values of the infested host plant, in terms of protein and sugar content, will have a direct effect on the performance (fitness) and developmental parameters of larvae that feed

inside the fruit (Kaspi et al., 2002). It is important to determine the differences in the development of these two species of fruit flies on different commercially grown host fruits in the Western Cape, in order to better predict which fruits would be preferred for oviposition and are at higher risk of being attacked, as this will help create an understanding of why the population numbers of the two species of fruit flies fluctuate the way they do, relative to each other, in certain areas. It was found in earlier studies that *C. captitata* does not distinguish future hosts on a fine scale basis, based on plant volatiles, but rather uses cues such as host size to differentiate between suitable hosts (Prokopy et al., 1984). Krainacker et al. (1987) found that *C. capitata* is an effective generalist in that it can utilize a number of different hosts by adjusting the individual values of various life history traits to maintain a generally high intrinisc rate of increase (r). This was also found by Carey (1984), who further established that the r value of *C. capitata* for stone fruit (particularly peaches) was higher than for pears, for example, with mango having an extremely high r value and net reproductive rate. However, very little/no information exists for *C. rosa* host utilization on deciduous fruits.

Two experiments were therefore done to determine how different commercial host fruits, grown in the Western Cape, affect the developmental parameters of the two fruit fly species. The aim of the first experiment in this chapter was to determine the acceptability of selected commercially grown deciduous and citrus fruit types to *C. capitata* and *C. rosa* and the influence that the different fruit types have on the egg development of these two fruit fly species. This was done by assessing the time it takes for eggs to hatch after being laid in different host fruits during no choice assays. The second experiment aimed to determine the effect of different commercial host fruits on developmental parameters of both species of fruit flies by looking at pre-adult success, in terms of pupal success (the number of larvae that

successfully develop into pupae), and adult success (the number of adults that successfully emerge from pupae), as well as adult fitness in terms of fecundity and longevity. The objective was to determine which hosts could be preferred by *C. capitata* compared to *C. rosa* due to developmental advantages. Such advantages will in turn lead to higher population numbers and a higher number of annual generations, which provide the opportunity for faster adaptation to the changing environment and improves survival. This information may improve understanding of the relative abundance and distribution of these two pest fruit fly species in the Western Cape, in relation to the fruit types grown in the region and the availability of different fruits during certain times of the year.

Materials and methods

Insect material

The adult fruit flies used in this study were obtained from the fruit fly colonies that were maintained in the insectary at the Department of Conservation Ecology and Entomology at Stellenbosch University. Colonies were kept in two separate rearing rooms, 2m × 3m at 12:12 (L:D) photoperiod and at a temperature of 25ºC (*C. capitata*) and 28ºC (*C. rosa*), respectively. Stock colonies were reared on banana, with protein and sugar also provided. Stellenbosch colonies originated from Citrus Research International, as outlined below. Adult females and males of *C. capitata* and *C. rosa* were caught from separate insect rearing cages (BugDorm-4030F; 32.5×32.5×32.5cm), in which they were being reared, using 13.5ml specimen glass vials with push-on lids to keep the flies inside. For the fruit inoculation experiment, all neonate larvae of *C. capitata* and *C. rosa* originated from colonies of the two species maintained at Citrus Research International (CRI) in Nelspruit, Mpumalanga Province, South Africa. These colonies were reared on artificial diet for about 200

generations. Flavour and previous experience with a host could affect the choice of larvae to feed on a host (Zucoloto, 1987; Canato & Zucoloto, 1998). The colonies are refreshed every 2 years whereby wild males obtained from fruit rearing or trapping are placed in cages with laboratory reared females.

Ceratitis capitata were identified by the scutellum which is mainly coloured solid black (Fig. 3.1).

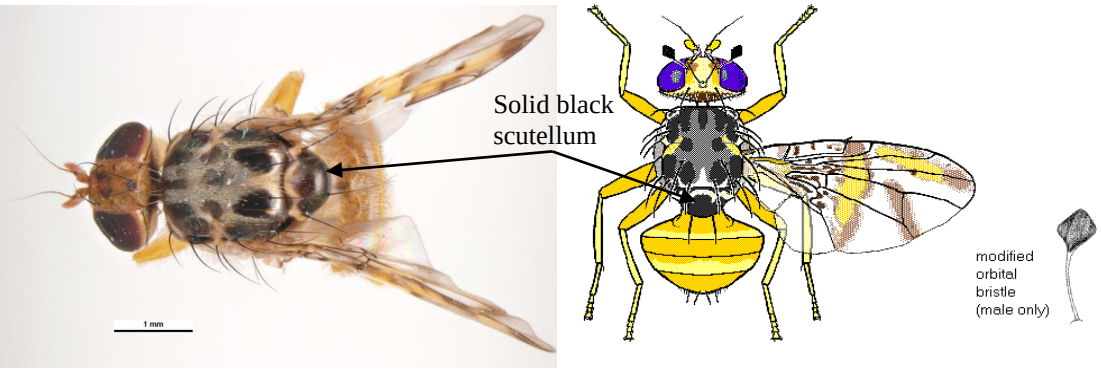

Figure 3.1: Female (left) and male (right) *Ceratitis capitata* identified by the solid black scutellum. (http://bugwoodcloud.org/images/768x512/5311093.jpg and http://delta-intkey.com)

The males were identified by the characteristic modified lower orbital setae which have black diamond shaped flags at the apex of the bristle and females were identified by looking for the absence of the lower orbital setae and the presence of an ovipositor (Fig. 3.1).

Ceratitis rosa were identified by the scutellum which is mainly coloured black, but have two creamy-white lines in the shape of a “n” separating the black colouration of the scutellum into three separate sections (Fig. 3.2).

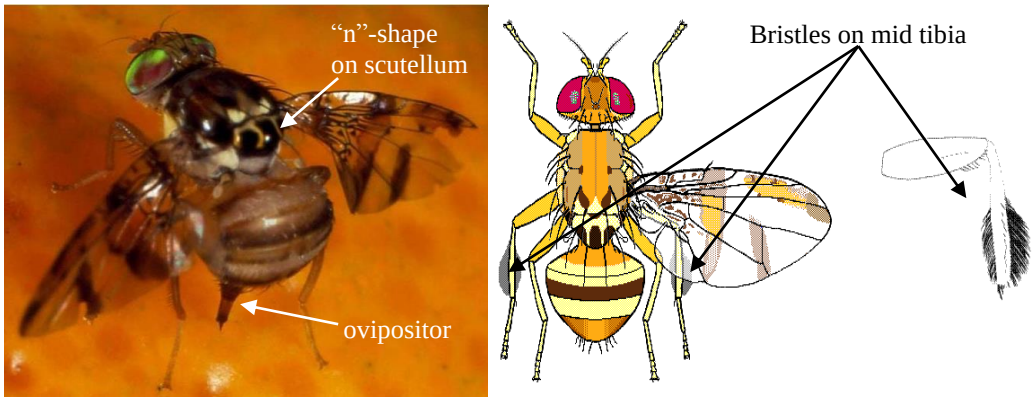

Figure 3.2: Female (left) and male (right) *C. rosa* identified by the "n"-shaped marking on the scutellum (http://www.cirad.fr and http://delta-intkey.com).

The males were identified by the dark coloured bristles that are present on the tibia of the middle pair of legs and the females by the absence of the bristles on the legs and the presence of an ovipositor (Fig. 3.2).

The flies of both species, at the time of catch, were 10 days old to ensure sexual maturity (Papadopoulos et al., 2002; Kaspi et al., 2002). For the 10 days prior to catching the flies for their use in the experiment, they were provided with water, protein hydrolysate and a sugar diet so as to not interfere with host preference during the experiment. A total of 120 females and 60 males were taken for each of the two fruit fly species.

At 10 days after adult emergence, 40 females and 20 males of the same species were then put into a BugDorm cage. Six types of fruit were tested: "Golden delicious" and "Granny smith" apples (*Malus domestica* L. Borkh.), "Crimson seedless" and "Dauphine" grapes (*Vitis vinifera* L.), "Excellence" peaches (*Prunus persica* Sieb. & Zucc.), "Packham's triumph" pears (*Pyrus communis* L.), "Angeleno" plums (*Prunus japonica* Thunb.) and "Navel" oranges (*Citrus sinensis* Osbeck). For apples and table grapes different cultivars were used, depending on availability in stores. Fruits evaluated were in the mature ripe stage of being fleshy and palatable and were acquired from Woolworths supermarket in Stellenbosch. One

fruit type was used per BugDorm cage. Three fruit units of each fruit type were inserted into the BugDorm cages to ensure that well over 100 eggs would be laid in the fruit. For grapes, two bunches each containing ≈ 50 berries were inserted as three grape berries would not have yielded a sufficient number of eggs. This was repeated five times for each type of fruit, with five separate batches of flies.

Fruits were rinsed in a 2% bleach solution prior to inserting them into the BugDorm cages, containing the flies, in order to sterilize against potential fungal or bacterial spores. The fruits were left to dry off on a cloth before being inserted into the BugDorm cages. Fruits were not manually punctured. The flies in the BugDorm cages were provided with some water by filling the bottom half of a 90mm Petri dish with water and some paper towel, that absorbed the water, inserted to prevent flies from dropping into the water and drowning. All BugDorm cages were then placed in a controlled environment room with a set temperature of 25°C and a 12:12 (L:D) photoperiod for 24h. For all replications of this experiment the BugDorm cages were subjected to the same environment controlled room, hence the same constant environment, as it was not possible to do all replications at the exact same time due to logistical difficulties.

Egg incubation

All fruits were removed from the BugDorm cages after 24 hours exposure to the ovipositing flies to ensure that the flies' eggs were as newly laid as possible without starting to hatch inside the fruit as it would then have been impossible to collect the eggs. Eggs of these two fruit fly species generally start hatching inside the host fruit at a temperature of 25°C after 48h. For this reason the fruit fly eggs were removed after 24h as the internal environment would have sufficiently influenced the egg development. If the internal environment of a fruit

were to be fatal to the fruit fly eggs, this would have also been apparent after 24h. Fruits were then dissected at the oviposition puncture sites using a scalpel and the fruit flies' eggs were extracted using a fine, no. 1 brush. A total of 100 fruit fly eggs were collected from each of the fruit types, for each of the two species of fruit flies, and placed on moist 90mm black filter paper in separate 90mm plastic Petri dishes. Black filter paper was used to more easily visualize the fruit fly eggs under a microscope. The Petri dishes were then marked with the date, species of fruit fly and fruit type from which the eggs were dissected, sealed with laboratory film (Parafilm M®) and incubated in a temperature controlled environment chamber (MRC model LE-509) at 25˚C with a 12:12 (L:D) photoperiod. The eggs were examined once every 24 hours for hatchlings until no further eggs hatched. The hatch rate could then be calculated for each fruit type as the percentage of eggs that hatched at the specific times for each of the replicates, by counting the number of fruit fly eggs that hatch out of 100.

Fruit inoculation

For the second experiment in this chapter, five types of commercial fruits were used to check for the effects the different fruits have on the development of the fruit flies, *C. capitata* and *C. rosa*, from their first instar larval stage through one life cycle. Fruits used were "Golden delicious" apples (*Malus domestica* L. Borkh.), "Packham's triumph" pears (*Pyrus communis* Linn.), "Crimson seedless" grapes (*Vitis vinifera* Linn.), Clementine (*Citrus unshiu* Swingle) and "Fan Retief" guava (*Psidium guajava* Linn.). These fruits were used due to availability during the time of the year and because each of these fruits are major commercial commodities produced in the Western Cape Province of South Africa.

Two thousand (2000) eggs per species were incubated on wet black filter paper per Petri dish in a temperature controlled environment chamber (MRC model LE-509) at 25˚C with a 12:12 (L:D) photoperiod, at the department of Conservation Ecology and Entomology, Stellenbosch. Petri dishes were sealed with laboratory film (Parafilm M®) to prevent the desiccation of eggs. After the eggs hatched, the different types of fruit were inoculated with neonate larvae to determine development with respect to each type of fruit.

Ten replicates of each type of fruit were completed. All fruits were weighed prior to the inoculation process so that pupal success could be expressed as the number of formed pupae per gram of fruit, and following further development, the number of adult flies that successfully emerged per gram of fruit. Fruits were rinsed in a 2% bleach solution in order to sterilize the outside of the fruit of any potential fungal or bacterial spores. After preliminary trial experiments conducted at CRI in Nelspruit, it was decided that 40 neonate larvae were the optimal number that should be placed into each fruit. Fourty neonate larvae were counted using a field microscope (Nikon, stereo microscope, model OBJ.2X) and placed in a drop of water on a Petri dish to prevent the larvae from moving away. Two holes, each with a diameter of 5mm, were carefully bored into opposite sides of the fruit to a depth reaching as near to the centre of the fruit as possible using a custom made hollow copper tube. Twenty larvae were then carefully placed into each hole as deep as possible using a very fine brush, therefore inoculating each fruit with the total of 40 larvae. The holes were then resealed by placing a small plug of cotton wool in each of the holes and then sealing it using molten wax (SASOLWAX 7835, Sasol Wax GmbH, Worthdamm 13-27 D-20457, Hamburg, Germany). The plug of cotton wool is to prevent any of the molten wax from moving down into the hole and potentially damaging the neonate larvae. This procedure was used for all fruits in all replicates of the present study except for grapes. Due to the smaller size and volume of grapes, four grape berries were used to represent one replication of grapes. One hole was

bored into each of the four grape berries where 10 neonate larvae were placed. Apart from this procedure, all replications were done in the same manner. The copper tube used to bore the holes in the fruits was sterilized after every inoculation using a 2% bleach solution to minimalize the risk of cross-contamination between individual fruits.

After being inoculated, each of the fruits was placed individually on a 2 – 3cm layer of sterilized sand in separate (5L) plastic containers (19×19×18cm). The sand was sterilized by placing it in a -15°C freezer for 48 hours. A large square opening was cut from each of the lids of the plastic containers and resealed by gluing grey organza on the inside of the lids. The material allows for light and air movement into the container and is fine enough to prevent flies from escaping. The sand was kept moist throughout the experiment by lightly spraying water through the organza of the lid of each container using a spray bottle. This was to simulate precipitation that would have occurred in the natural environment. The sand in each of these containers was sieved every second day to check for pupae.

All replicates were kept in an environment controlled room with a set temperature of 25°C and a 12:12 (L:D) photoperiod.

Pupal success

Pupae that were recovered during the sieving were placed on a 1cm layer of sterilized sand in separate specimen jars (40ml), with punctured lids for ventilation. Each replicate had a specimen jar in which pupae were placed. The specimen jars were checked on a daily basis for adult fly emergence and the sand in each jar was kept moist to prevent the potential desiccation of the pupae. Care was taken as to not saturate the sand with water in which case the pupae may drown or their development may be negatively affected due to a lack of air. Adults that emerged were counted and sexed using the same identification method as

explained earlier in the insect material section of the first experiment in this chapter. Specimen jars were incubated in the same environment controlled room with a set temperature of 25°C.

Fecundity and longevity

As soon as an emerged adult male-and-female pair from one type of fruit became available, they were removed from the specimen jars and placed in a separate plastic container (19×19×18cm). The containers used here were the same as the ones used for placing of the inoculated fruits, with organza in the lids to allow for light and ventilation. Each adult pair was kept at 25°C in a separate container and provided with fresh water, sugar (Behar et al., 2008) and protein hydrolysate throughout the experiment. After ten days, when the female fruit flies had reached sexual maturity, a glass vial with a small 5×5mm slice of guava was introduced on top of each of the containers. The lids of the vials were punctured to allow the guava scent to escape, which should stimulate the females into laying eggs through the organza. This was done for each of the adult pairs irrespective of which type of fruit they had emerged from. The lid of each container was checked on a daily basis to collect any eggs that had been laid. Eggs were collected from the organza using a fine no. 1 brush and were placed on wet black filter paper in a Petri dish. The total numbers of eggs laid were counted in order to calculate fecundity of female fruit flies with respect to the type of fruit they had developed in. Adult pairs were kept in the containers and monitored until 100% mortality occurred.

Statistical analysis

A repeated measures ANOVA was used to compare egg hatch between fruit fly species and fruit types (Statistica 10, StatSoft Inc., 2013). Interactions between fruit fly species and fruit

types were also determined. No egg hatch was observed at the 24h time interval. The 24h time interval was thus excluded from statistical analyses due to the high occurrence of zero values recorded for this time interval, skewing the data. After the exclusion of the 24h time interval, a chi-square test was performed and data were found to be normal (Chi = 310.29; df = 9; $p < 0.001$).

A factorial ANOVA was performed to determine effect of fruit type on the development of the two fruit fly species, as the data fitted the assumption of normality. Fruit fly species and fruit type were used as catergorical variables, while number of insects (pupae, adults) per 100g fruit was the dependant variable. For fecundity (unbalanced design and limited fruit types), a non-parametric test was used. A Mann-Whitney U test was done on the number of eggs laid per adult female per day in order to determine potential differences in fecundity between the two species of fruit flies that were brought on by developing in different fruit types. All statistical analyses were done using Statistica 10, StatSoft Inc. (2013). The survival of both species of fruit flies was determined by starting with a figure of 100 percent, on day one of adult emergence, and then monitoring daily to determine the percentage survival. For example, where twenty *C. capitata* adults were used to represent the total population of adults that emerged from guavas, each individual represented five percent.

Results

Egg development

No significant differences in the egg hatch of the two species of fruit flies were found between the different types of fruit. No positive puncture response was found on oranges so this fruit type was excluded from the analysis. The interaction between the fruit type and the species of fruit flies was not significant and did not affect the percentage egg hatch ($F_{(4, 40)}$ =

0.448, p = 0.773) (Fig. 3.3). Although not significant, egg hatch was greater for *C. rosa* on pome fruit, while *C. capitata* egg hatch was greater on stone fruit, with grapes yielding more or less similar egg hatch.

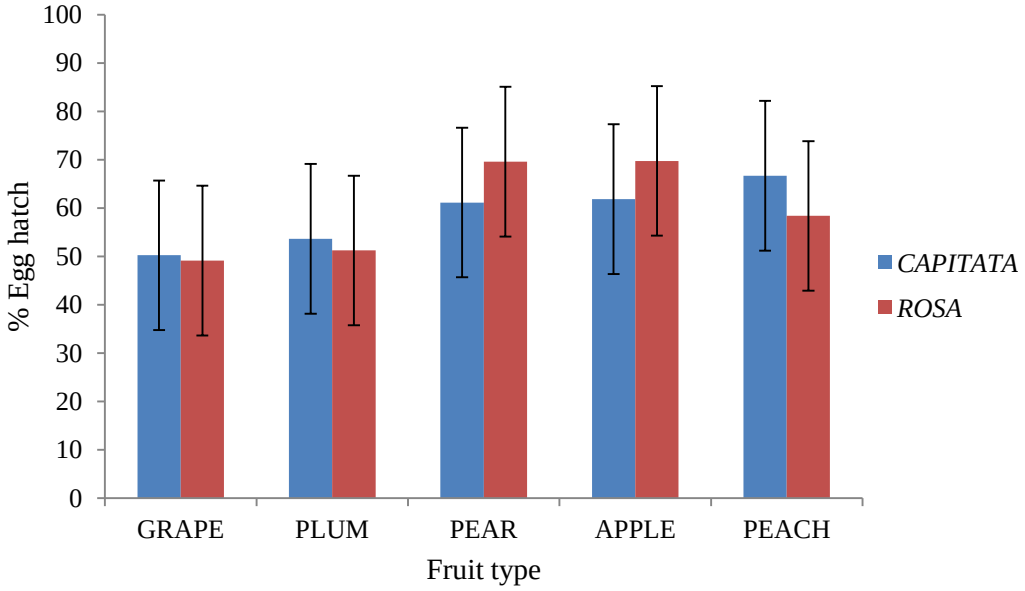

Figure 3.3: Percentage egg hatch of two *Ceratitis* species on five deciduous fruit types assessed during laboratory experiments. Error bars denote 95% confidence intervals.

Time had a significant effect ($F_{(3,\ 120)} = 150.17$, $p < 0.001$) on the number of eggs that hatched, regardless of the type of fruit or the species of fruit fly. The results indicate that for a wide range of host plants, more than 50% of all the eggs laid for both *C. capitata* and *C. rosa* will hatch within the first 72 hours at 25° C. Around 90% of all eggs laid would have hatched by 168 hours (Fig. 3.4).

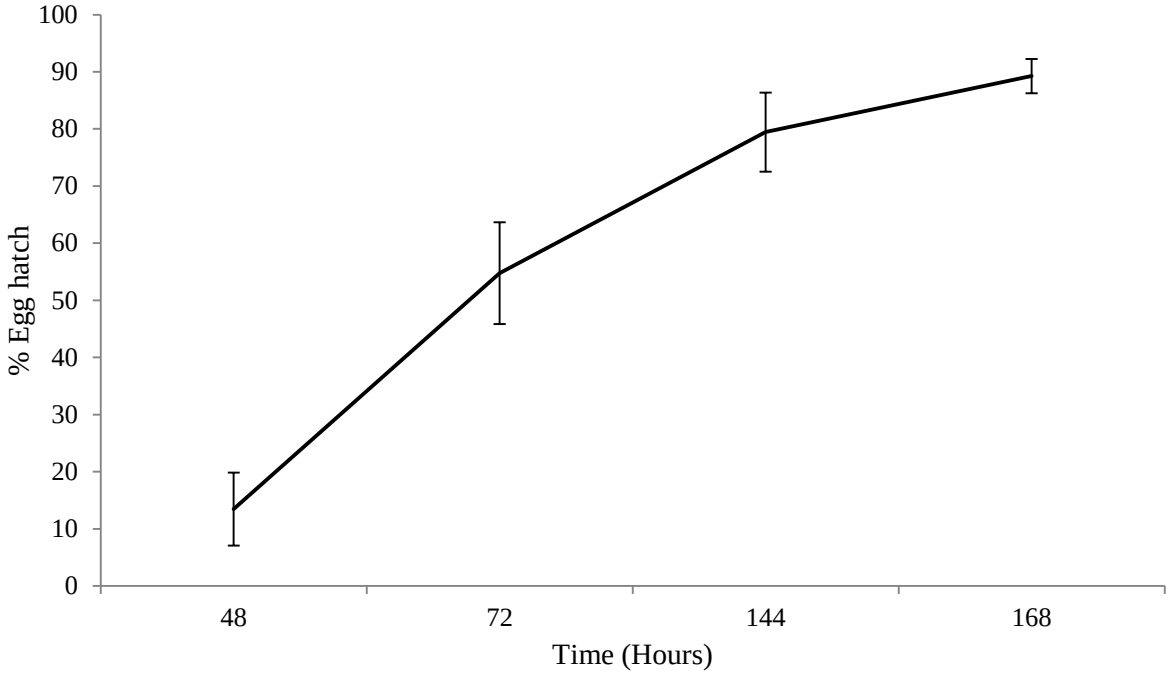

Figure 3.4: The combined percentage egg hatch of two species of fruit fly, *Ceratitis capitata* and *C. rosa*, on apples, grapes, peaches, pears and plums, assessed during laboratory experiments. Error bars denote 95% confidence intervals.

Larval development

Generally, development of *C. rosa* and *C. capitata* larvae took longer in pome fruit (apple and/or pear) compared to other fruit types (Table 3.1). Larval developmental times for both *C. capitata* and *C. rosa* were shortest in table grapes (Table 3.1). The number of days for larval development of *C. rosa* in apples were significantly more than the number of days required by *C. capitata* in grapes ($p = 0.049$). The number of days required for the larval development of *C. capitata* larvae in guava was significantly less than the time required for *C. rosa* larvae in apples ($p = 0.013$). The number of days for larval development of *C. rosa* was significantly less ($p = 0.014$) for larvae in guavas compared to apples (Table 3.1). No pupae were recovered from apple for *C. capitata* during the eleven weeks following the inoculation of fruit with larvae.

Table 3.1: The average number of days required for larvae of *Ceratitis capitata* and *C. rosa*, developing in different host fruits, to reach pupation.

SPECIES	Apple	Clementine	Grape	Guava	Pear
C. capitata	0.0	18.68	13.87	16.28	21.63
C. rosa	24.51	17.84	13.84	16.57	19.81

Pupal success

For the number of pupae that emerged, there were significant interactions for the species of fly emerging from all fruits combined ($F_{(1, 90)} = 4.175$, $p < 0.05$), with *C. capitata* yielding significantly more pupae than *C. rosa*. The effect of fruit type on pupae emerging was highly significant ($F_{(4, 90)} = 30.311$, $p < 0.001$), with guava yielding significantly more pupae than any of the other fruits (Fig. 3.5). The interaction between fruit type and species of fly was also significant ($F_{(4, 90)} = 2.788$, $p < 0.05$). For both species of fly, guava yielded significantly more pupae than any of the other fruit. Apple, clementine and pear yielded the least numbers of pupae. The numbers of *C. capitata* pupae on grapes were intermediate. *Ceratitis capitata* yielded more pupae only for guava and grapes (Fig. 3.5).

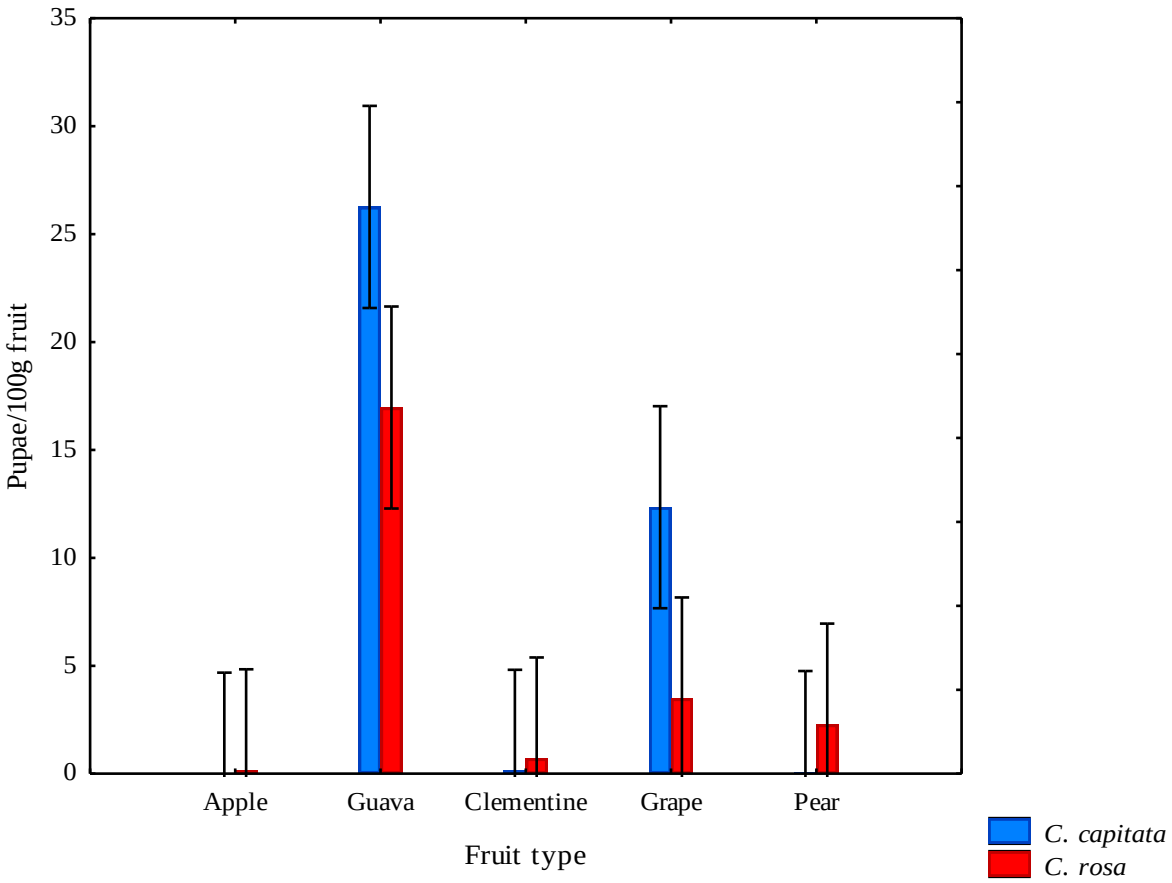

Figure 3.5: The number of *Ceratitis capitata* and *C. rosa* pupae, recovered from different types of fruit during laboratory experiments. Error bars denote 95% confidence intervals.

Adult success

The effect of fruit type on adult emergence was gender specific. There was no significant difference between species when considering the number of males emerging from all fruits ($F_{(1, 90)} = 0.206$, $p = 0.65$). The interaction between fruit type and species of male flies was not significant ($F_{(4, 90)} = 2.261$, $p = 0.69$). The effect on fruit type was highly significant for males ($F_{(4, 90)} = 18.349$, $p < 0.001$), with guava yielding significantly more pupae than all other fruit types (Fig. 3.6).

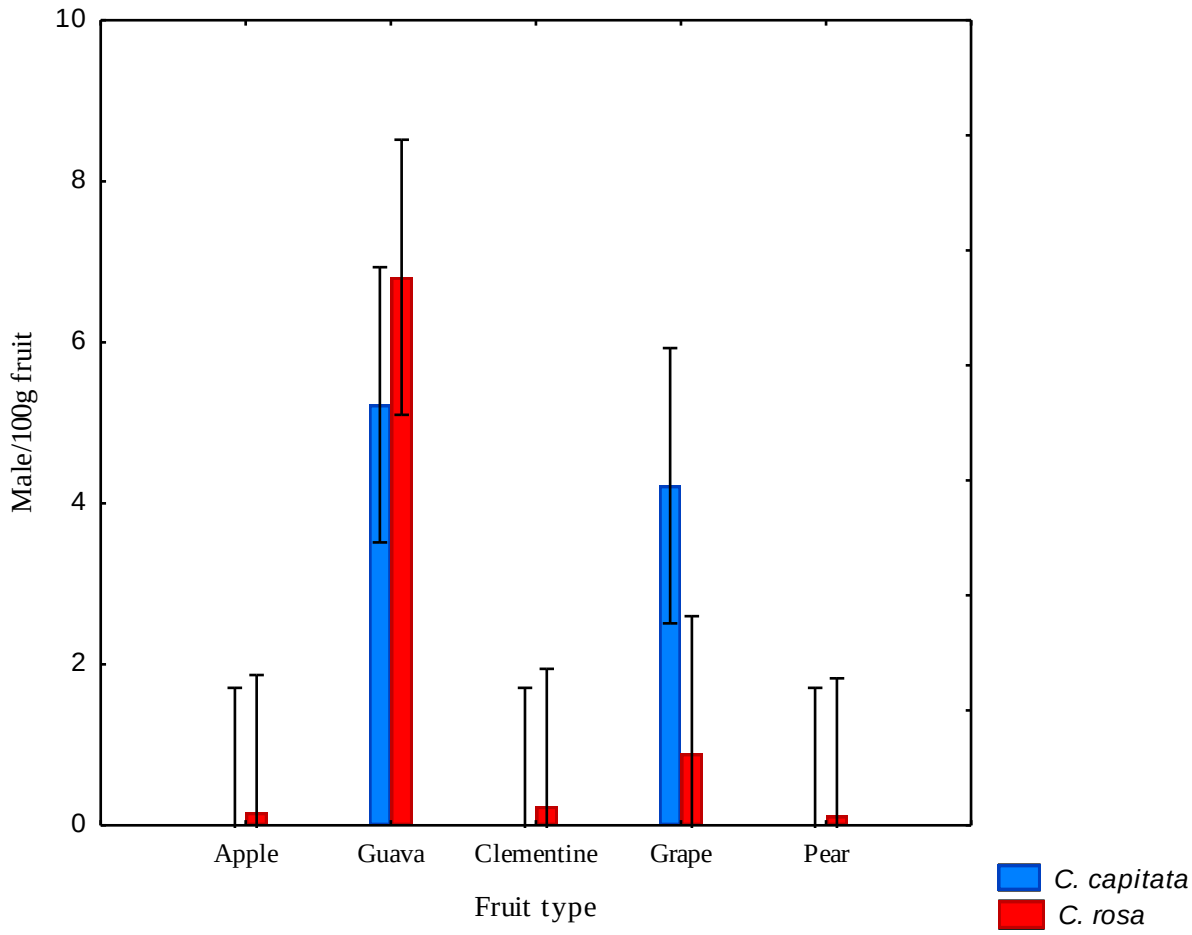

Figure 3.6: The number of *Ceratitis capitata* and *C. rosa* males recovered from different types of fruit during laboratory experiments. Error bars denote 95% confidence intervals.

With females, more *C. rosa* emerged from all fruits combined than *C. capitata*, although this was not significant ($F_{(1, 90)} = 2.969$, $p = 0.09$). This differs from what was observed for pupae and males, where *C. capitata* was dominant. The effect of fruit type on female emergence was also highly significant ($F_{(4, 90)} = 28.145$, $p < 0.001$), and as with males, guava yielded significantly more females than all other fruit types (Fig. 3.7). Lastly, again the interaction between fruit type and species of fly was significant ($F_{(4, 90)} = 2.875$, $p < 0.05$), with *C. rosa* emerging with significantly more flies than *C. capitata* on guava, and guava yielding significantly more flies than all other fruit types (Fig. 3.7).

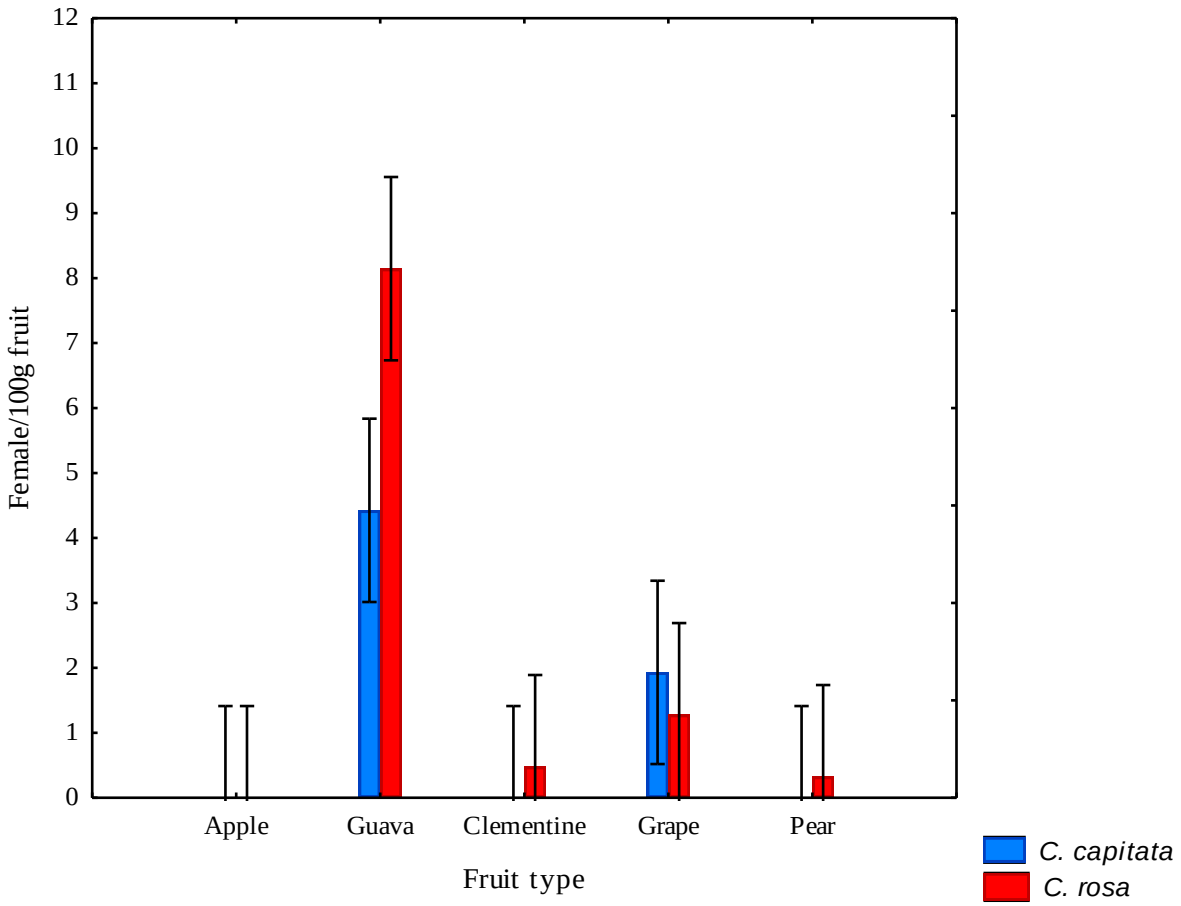

Figure 3.7: The number of *Ceratitis capitata* and *C. rosa* females recovered from different types of fruit during laboratory experiments. Error bars denote 95% confidence intervals.

It is further interesting to note that *C. capitata* adult emergence on apple, clementine and pear was zero or negligible. *Ceratitis rosa* was also not successful on apple, except for males and a small number of pupae (Figs. 3.5, 3.6 and 3.7).

Fecundity and longevity

Adult male-female pairs could only be acquired for *C. capitata* that emerged from grapes and guavas. Individual adult flies emerging from the other fruits tested in this chapter emerged at different times so that it was not possible to group other adult pairs together for testing fecundity. The ten adult pairs of *C. rosa* that were obtained from guavas, did not lay eggs through the organza lids of the experimental tubs, and therefore fecundity for *C. rosa* could

not be determined. *Ceratitis rosa* male-female pairs were however kept to check the longevity of the species.

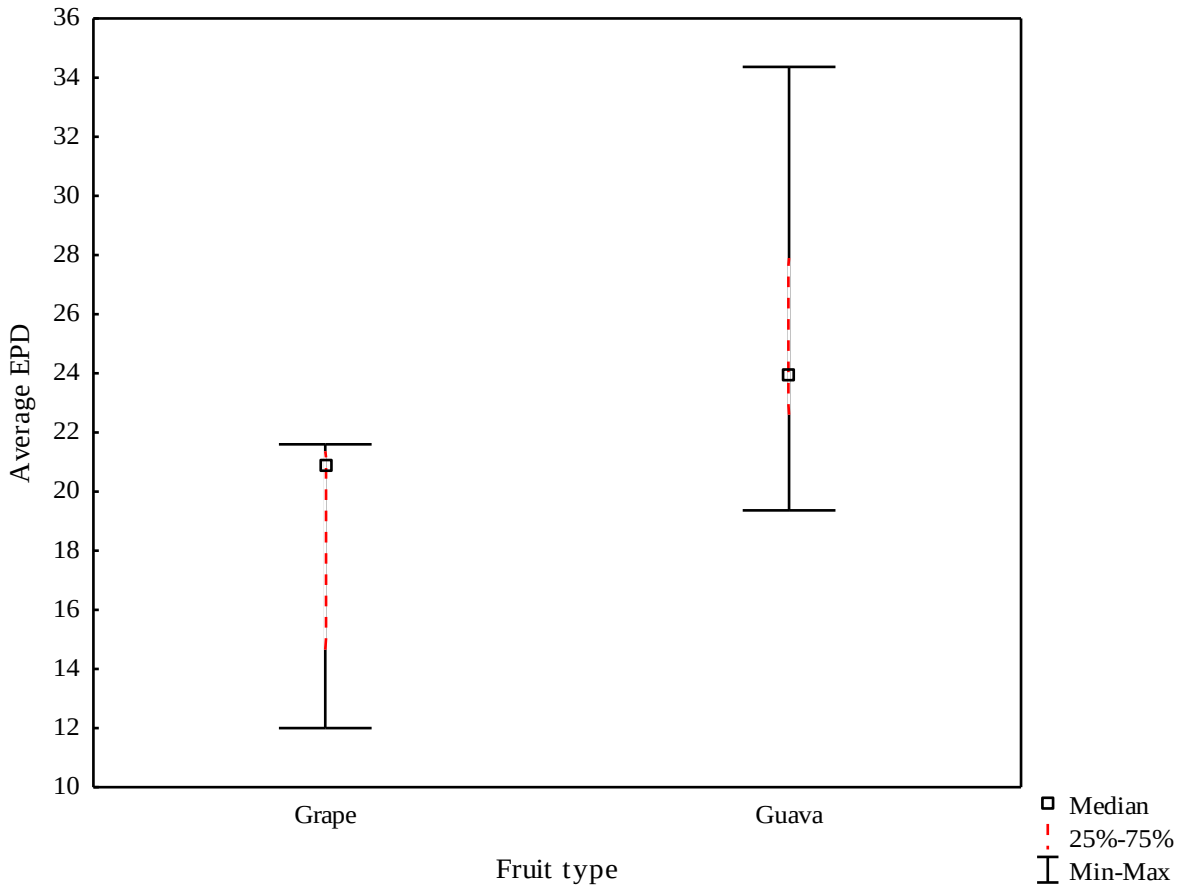

Figure 3.8: The range of the average number of eggs that were laid per *Ceratitis capitata* female per day (EPD) for two different fruit types.

Females that emerged from pupae recovered from guavas, laid significantly more eggs per day compared to females that emerged from grapes ($p = 0.023$) (Fig. 3.8). Females that emerged from guavas and grapes, on average, laid 24.98 and 18.10 eggs per female per day (EPD), respectively. Females that emerged from guavas and grapes, on average, had a gross fecundity of 1208 and 1150.4 eggs per female during their lifetime, respectively. The average number of days that females emerging from guavas and grapes survived was 51.3 and 60 days, respectively.

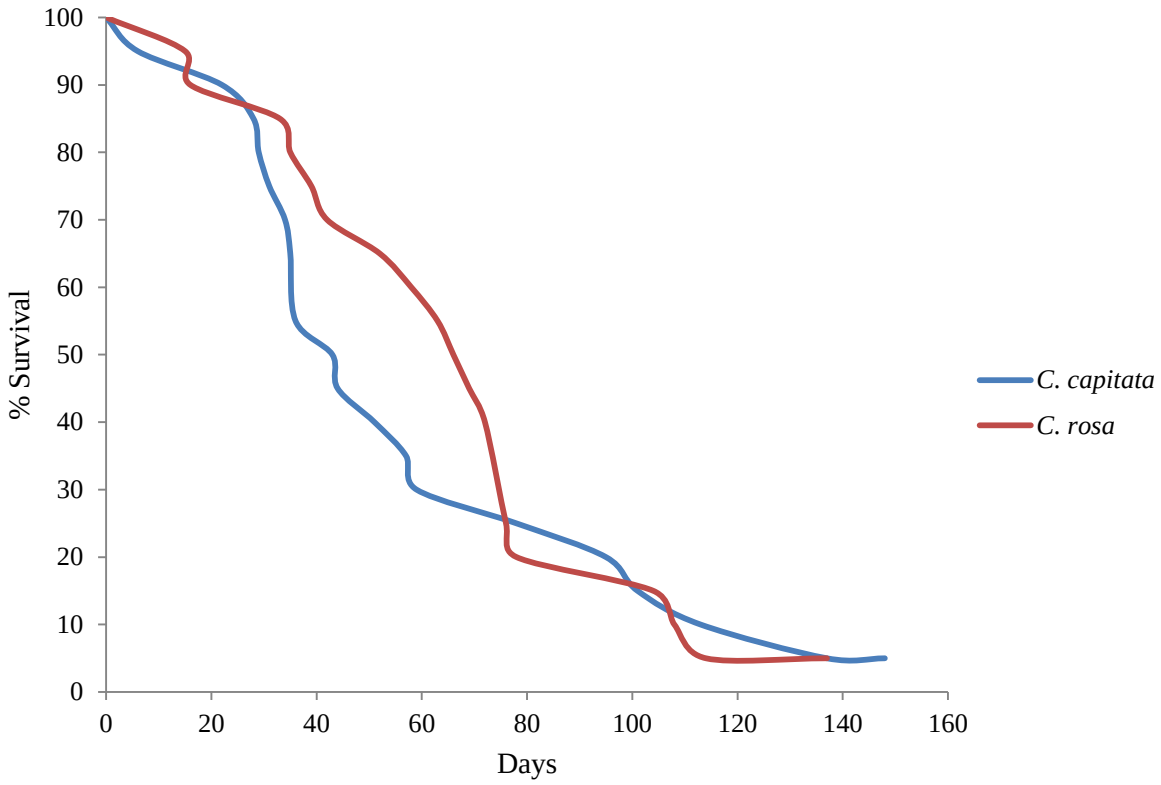

Figure 3.9: Survivorship of ten male and ten female *Ceratitis capitata* and *C. rosa* adults that were reared on guavas during laboratory experiments.

Ceratitis capitata adults reared on guavas lived for up to 148 days with 50% of the population remaining alive after 43 days, whereas adult *C. rosa* reared on guavas lived up to 137 days with 50% of the population remaining alive after 66 days (Fig. 3.9). A small number of flies from both species were still alive at the time of writing up this chapter.

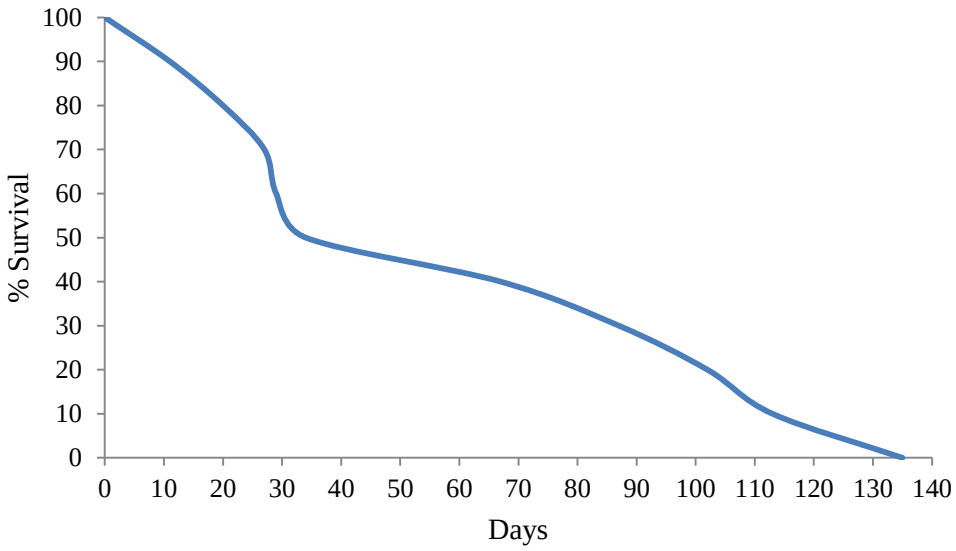

Figure 3.10: Survivorship of five male and five female *Ceratitis capitata* adults that were reared on grapes during laboratory experiments.

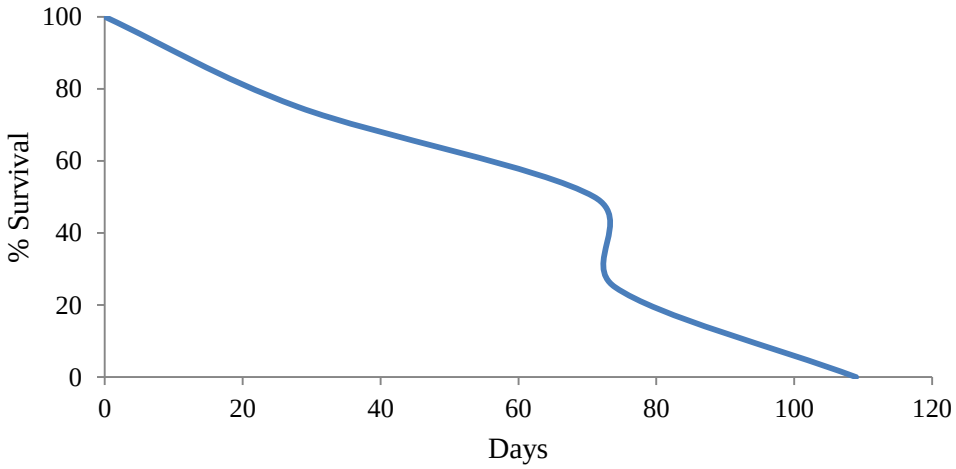

Figure 3.11: Survivorship of two male and two female *Ceratitis rosa* adults that were reared on grapes during laboratory experiments.

Some *C. capitata* adults reared on grapes survived for a total of 135 days and *C. rosa* adults reared on grapes survived 109 days (Fig. 3.10 and Fig. 3.11). *C. capitata* males and females reared on guavas were found to live an average of 46.22 and 61.90 days, at 25°C, respectively. *Ceratitis capitata* males and females that were reared on grapes were found to

live an average of 53 and 72 days, respectively. *Ceratitis rosa* males and females reared on guavas lived an average of 55.11 and 69.60 days, respectively, while males and females reared on grapes lived 51 and 71 days, respectively. Some of the early deaths were due to flies that got stuck in the protein hydrolysate diet.

Discussion

The present study found a clear preference for guava for both fly species, in terms of immature development and adult rearing success, survival and fecundity, with the opposite true for apple and clementines. José et al. (2013) reported guavas to be the fruit type most infested by *B. dorsalis*, *C. capitata* and *C. rosa* during a study in Mozambique. They also found the number of pupae/fruit and pupae/kg of fruit to be higher on guavas than other fruit types. White & Elson-Harris (1992) reported that *C. capitata* selectively attacks oranges and that they would oviposit in oranges that had fallen from the tree and are damaged. Further studies conducted on different orange varieties with *C. capitata* found no differences in egg hatch and incubation period between sweet orange, bitter orange and lemon varieties, although it was thought that *C. capitata* was not well adapted to citrus species as the female ovipositor may only reach into citrus rind, where high mortality of immature stages was recorded (Papachristos et al., 2008). Low infestation rates were found for all citrus observed during a study by Mwatawala et al. (2009) and they described citrus as being a poor host of fruit fly pests in Tanzania. Muthuthantri & Clarke (2012), during a study on the citrus hosts of *Bactrocera tryoni* (Froggatt), found a negative correlation between the toughness of the peel and *B. tryoni* preference for oviposition. Carey (1984) found a positive puncture response for *C. capitata* on apple (*Malus sylvestris*), but no pupae emerged during his experiments. Mwatawala et al. (2009) sampled a small number of apple (*Malus domestica* Borkh.) from the field in Tanzania with only *C. rosa* emerging. Fruit infestation of apple (*M.*

domestica) was intermediate, compared to stone fruits, in a study comparing management practices in different fruit production areas, where all fruits were infested with *C. capitata* (Manrakhan & Addison, 2014). The fact that no *C. rosa* were found from these fruits could, however, be a factor of geographical location (climate) as well, as apples were only sampled in areas where *C. capitata* was dominant (Manrakhan & Addison, 2007). Again in Tanzania, *C. rosa* was more frequently sampled on temperate fruits (apple, pear and peach) and was generally dominant over *C. capitata* (Mwatawala et al., 2009), indicating the influence of climate. Aside from guavas, table grapes were found to be the most suitable host for both fruit fly species form the current study. Previous studies of *C. capitata* found grapes (*Vitis vinifera*) to be less suitable hosts compared to pome and stone fruits (Carey, 1984; Mwatawala et al., 2009).

Shoukry & Hafez (1979) found the same time required for egg hatch during laboratory experiments with *C. capitata* than the present study, although these results are not comparable to a study done by Krainacker et al. (1987), where they assumed that 97% of *C. capitata* eggs hatch within 48 hours at 25°C and that this was constant independent of the type of host in which the eggs were laid during laboratory experiments. Shoukry & Hafez (1979) further found that the temperature greatly affects the duration of the egg stage, with 25°C being the more optimal temperature compared to 31°C for *C. capitata*. Since all replications of the present study were kept at a constant temperature of 25°C the temperature in the present study was not a factor for consideration for any differences observed in egg hatch or immature development. However, in the present study, no significant differences were found in egg development for any of the fruit types for either fly species. These results indicate the polyphagous nature of these pest species and how they have evolved to develop in a wide range of host plants (Krainacker et al., 1987), and that all fruit types evaluated here were suitable for the egg development of the two fruit fly species. The results in this study

further indicate that the fruit type had no effect on the egg hatch of both species of fruit flies. *Ceratitis rosa* shows the same pattern of egg hatch as *C. capitata*, where eggs are resilient and able to develop in a range of different fruit types. According to Aluja & Mangan (2008) natural hosts (fruit or vegetables found infested under natural field conditions) of Tephritidae are not always suitable for the development of the larvae and that non-natural hosts (fruits or vegetables infested under laboratory conditions) are sometimes more suitable for the development of the larvae. There are many factors that could lead to adult females laying eggs in non-natural hosts in nature or under laboratory conditions, including the host quality, genetics, learning, potential fecundity, ovarian dynamics, female age, social context, chemical context and individual variation in ovipositing decisions (Aluja & Mangan, 2008). The influence of natural vs. non-natural hosts on fruit fly development was not tested in the present study.

The present study found that larval development for both species was fastest in grape, followed by guava, clementine, pear and apple, with no development found in apple for *C. capitata*. *Ceratitis rosa* mostly developed faster than *C. capitata* except in guava, where development time was approximately the same. For prune, pear and apple, minimum egg and larval development was faster for *C. capitata*, while for guava and plum *C. rosa* development was faster and on peach both were approximately equal in development time (Myburgh, 1956). Carey (1984), who studied demographic parameters of *C. capitata*, found that larval development was fastest in nectarine, followed by orange, plum and peach, pear and lastly apple, with highest percentage survival in peach and the lowest in grape. This is comparing only the temperate fruits tested during the previous study. These variable results could have been influenced by the cultivar of fruit used. In the present study, significantly more *C. capitata* pupae were recovered from guavas compared to *C. rosa*, but *C. rosa* females had a significantly higher percentage adult eclosion compared to *C. capitata* on guavas. This was

not the case for males, where more (but not significantly more) male *C. capitata* emerged, as with pupae. In general, the results of the present study indicate high mortality (failure to eclose) of *C. capitata* compared to those of *C. rosa*, while in the study by Carey (1984) survival of pupae was generally high (from 73 to 100%). This could be due to laboratory conditions, where it is hard to accurately replicate the natural conditions under which these fruit flies would usually develop. The amount of time that the fruits were kept in cold storage and its effect on the quality of the fruits, the type of post-harvest treatment the fruits underwent and possible insecticide residues on the skin of the fruits could affect the outcome of development for such pest species as *C. capitata* and *C. rosa* (Drogué & DeMaria, 2012; Iizuka et al., 2013). This was not accounted for in the present study and the experiments could be improved by potentially utilizing organic fruits where management protocols are available and which are picked directly from the host plant for the experiments. In-field experiments would further account for changes in nutrition occurring once the fruit are picked, but could be difficult to quantify.

Females in the present study had a gross fecundity of up to almost double the amount, and longevity of almost twice the number of days, that were reported in previous demographic studies of *C. capitata* (Shoukry & Hafez, 1979; Carey, 1982; Krainacker et al., 1987). Krainacker et al. (1987) reported an average of between 8.5 and 19.7 EPD, and gross fecundity of *C. capitata* females to be between 490 and 690 eggs. Carey (1982) found that females laid 26 eggs/ 2 days (13 EPD) during peak reproduction. Shoukry & Hafez (1979) found that female *C. capitata* had a gross fecundity of 826 eggs, and that females lived an average of 31 days at 25°C. The high gross fecundity in the present study could be due to the longevity of the females, which lived almost twice as long as compared to the study by Shoukry & Hafez (1979), although Krainacker et al. (1987) suggests that high fecundity was

due to a higher daily egg production and not the number of days that the females survived. Ferandes-da-Silva & Zucoloto (1997) reported that *C. capitata* females that feed on the apical portion of papayas, where higher sugar content existed compared to the basal portion of the fruit, had more developed ovaries and in turn had a higher fecundity. Additionally, females of *Anastrepha* spp. that had fed on both sucrose and protein had greater egg loads than those that fed on sucrose alone (Aluja et al., 2001). Harwood et al. (2013) described that poor dietary conditions can lead to a reduced reproductive effort in order to allocate resources into surviving until food availability improves again. In the present study, the number of days that female *C. capitata* survived could be higher for females reared on grapes due to a lower fecundity in terms of EPD. Chapman et al. (1998) described that there is a cost associated with reproduction in many insects and that virgins were found to live longer than non-virgins, or less frequent maters lived longer than frequent maters.Krainacker et al. (1987) suggest that a reason why *C. capitata* is such a successful generalist is because the species is capable of compensating certain life-history traits when developing on different hosts such as fecundity and survivorship, or development time and larval survival.

The remaining fruit types used in the present study should still be considered as important hosts for the two species of fruit flies as the availability of hosts vary during certain times of the year. In the case of the two studied fruit fly species, the population numbers will spike in an area where preferred hosts become abundantly available during fruiting seasons (De Villiers et al., 2013). Host availability is one of the most important factors driving the relative abundance and distribution of fruit fly pests, as they synchronize their emergence close to that of the fruiting phenology of their hosts (Christenson & Foote, 1960; Carey, 1984; Aluja & Mangan, 2008; Mwatawala et al., 2009; Vayssières et al., 2009). José et al. (2013) reported that although *B. dorsalis* (previously *B. invadens*) pupae emerged more frequently from

tropical almonds compared to other hosts, the adults that emerged showed no difference in the weight, size and flight ability compared to all other hosts observed. From this we could deduce that even if certain fruits consistently yield more flies, this does not mean that they are better in terms of weight, size and fitness, but that this could just be due to what hosts are available at the time.

The results in the present study are a first step to understanding different life-history parameters of *C. capitata* and *C. rosa*, on different hosts, in the Western Cape. This will enable management programmes to rank hosts according to their infestation status by different fruit flies. In host fruits where the number of days required for larval development is high, the host could be viewed as a potential host in which the fruit fly species can overwinter and survive during unfavourable conditions (Papadopoulos et al., 2002). Such a host could also provide refuge to the fruit flies during times when host-availability is low. *Ceratitis rosa* have been found to displace *C. capitata* populations when the climatic conditions were less favourable for *C. capitata* (De Meyer et al., 2008). Where *C. capitata* and *C. rosa* may develop equally well in a certain hosts, under standard conditions, the outcome may vary according to different climatic conditions. Duyck et al. (2006) describe that in Reunion *C. capitata* dominate in ranges of 24 - 26°C, 0 – 1000mm rainfall while *C. rosa* populations peak in ranges of 22 - 23°C, 3000 – 3500mm rainfall. *Ceratitis capitata* and *C. rosa* populations segregates ecologically and geographically according to these ranges, and these two species are able to coexist in areas due to climatic niche differentiation (Duyck et al., 2006). The same could be true in South Africa, as the present study found no clear differences in host utilization between the two species of fruit flies and that both were able to complete their development on all hosts studied. However, De Villiers et al. (2013) found *C. capitata* to be widely distributed throughout South Africa, while *C. rosa* was very limited/absent in drier regions. Under optimal climatic conditions for both species, these two

flies would be able to develop equally well on fruits available at the time. While the temporal distribution of *C. capitata* and *C. rosa* is affected by availability of hosts, the spatial distribution of the two species is likely to be influenced by factors other than host type such as temperature and rainfall.

Conclusion

The fruit types that were used in the present study are acceptable by both fruit fly species. Both species of fruit fly did not lay eggs in oranges, but this finding could not be used to extrapolate on the non-acceptance of oranges, as laboratory studies on picked fruit might not reflect the behaviour under more natural conditions. Different fruit types are suitable for both species of fruit flies, and the two species did equally well on the fruit types studied here. Development of all immature stages could be completed in all fruit types. In the present study, guavas were found to be the host in which the larval stages of *C. capitata* and *C. rosa* experienced the highest developmental benefits and that these flies will more readily target this host where it occurs in the field (Mwatawala et al., 2009; Rwomushana et al., 2008). Areas where guavas occur, whether it is on a commercial or non-commercial scale, should be included into the area-wide management programmes of these two fruit fly species. A further focus should be the management of fruit flies on table grapes, as this proved to be a suitable host for fruit flies, as table grapes are one of the main commercial export fruits from the Western Cape Province. There were no differences in host utilization between *C. capitata* and *C. rosa*, demonstrating that if climatic conditions were optimal for both species, they would be able to develop in most deciduous hosts available at the time. While the temporal distribution of *C. capitata* and *C. rosa* is affected by availability of hosts, the spatial

distribution of the two species is likely to be influenced by factors other than host type such as temperature and rainfall.

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Chapter 4: Wing shape variation in *Ceratitis capitata* (Diptera: Tephritidae) fruit flies reared on three different host fruit

Introduction

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) is highly polyphagous and is an economically important pest of fruits, vegetables and nuts. *Ceratitis capitata* has become widely distributed throughout the world possibly through trade of fruit and vegetables (José et al., 2013; Mwatawala et al., 2009; IAEA, 2003). It is important to gain a better understanding of the status of this fruit fly species on various host plants in order to better manage their population numbers and in turn the damage that they cause on commercial and non-commercial host plants.

The quality and quantity of food available to the fruit flies during their immature stages play a major part in their development and ultimately the fitness, size and shape of the adults (Canato & Zucoloto, 1998; Badyaev et al., 2005). Studies on the size and shape of species are being used in order to establish traits associated with their fitness and development, and how internal factors such as nutrition (Kölliker-Ott et al., 2003; Kitthawee & Dujardin, 2010) and external (e.g. environmental) factors (Bomfim et al., 2011) cause morphological variations.

Morphological evolution explains that a certain genotype can express different phenotypes depending on the surrounding environment the organism develops in (Charlesworth et al., 1982; Smith et al., 1985). Individuals in populations may experience environmental and/or genetic disturbances during their development which influences the normal development of such individuals. These developmental disturbances are often expressed as a morphological trait of these individuals, such as asymmetry (Ludoški et al., 2012). Ludoški et al., (2012) describes three kinds of asymmetry namely, directional asymmetry, fluctuating asymmetry

and antisymmetry. Fluctuating asymmetry can be used as an indicator for whether an individual experienced stress during its development, or not (Ludoški et al., 2012).

The *Drosophila* wing is an excellent indicator of morphological evolution of species in this genus and the size of their wings have been described as a highly plastic trait that reflects the developmental influences of being exposed to different environmental conditions (Soto et al., 2007). Evidence exists that natural selection targets both wing size and shape (wing morphology) as different variations of form have been recorded for the same species on different continents (Soto et al., 2007). Morphological evolution explains that a certain genotype can express different phenotypes depending on the surrounding environment the organism develops in (Charlesworth et al., 1982; Smith et al., 1985).

Morphometrics is used to describe and compare shapes of organisms or specific structures that could be brought on by factors such as geographical location, environmental influences, developmental stages and genetics (Rohlf & Marcus, 1993). Traditional morphometrics will make use of wing measurements such as wing length, width and area in order to calculate variation in wing shape for a certain species.

Geometric morphometrics uses methods that include using a set of standardized landmarks on a morphological structure, such as specific intersections of veins in the wing of a certain insect, in order to statistically describe and analyse variations of shape within and among samples of organisms (Rohlf & Marcus, 1993; Rohlf, 1999). Kitthawee & Dujardin (2010) describe geometric morphometrics as an informative tool which can be used to describe intraspecific variation brought on by host plants or other factors that influence the morphology of the *Bactrocera tau* complex. Bomfim et al. (2011) found geometric morphometrics to be more sensitive in studying morphological variation in *Anastrepha pickeli* Lima populations from different localities than standard morphometric measurements.

Schutze et al. (2011) describe that geometric morphometric analysis can be used to resolve fine scale differences in the *B. dorsalis* complex, and that it can be used as a rapid identification tool when a broad, comprehensive dataset is available. Stark et al. (1999), in a study of the evolution of dipteran wing veins, described the necessity of comparative studies of the morphology of different dipterans in order to advance our understanding of the molecular basis of morphological variation. Klingenberg & McIntyre (1998) studying *Glossina palpalis gambiensis* (tsetse fly) wings using geometric morphometrics, demonstrated that the variation in the patterns of the wing landmarks can be interpreted in terms of differences in development, and that it is crucial to understanding the developmental basis of morphometric variation.

The present study makes use of geometric morphometrics to determine whether differences in the shape of the wings occur when *C. capitata* flies are reared on three host fruits, namely pome fruit (pears), stone fruit (plums) and citrus (clementines). Differences in the shape of the wings should reflect differences in the development of these fruit flies on various host fruits due to the presence of more, or less, optimal nutrition in these hosts. The objective of this study was therefore to determine if this new method would be able to detect slight changes in wing morphology relative to the development of *C. capitata* in different host fruits.

Materials and methods

Rearing adults

Three host fruit were tested in this experiment in order to compare the differences in development of adult *C. capitata* when reared on these fruits. The three hosts were: stone fruit “Angeleno” plum (*Prunus japonica* Thunb.), pome fruit “Packham’s triumph” pear

(*Pyrus communis* Linn.) and citrus clementines (*Citrus unshiu* Swingle). *Ceratitis capitata* pupae were acquired from the Citrus Research International (CRI) colonies in Nelspruit. 4000 pupae were divided into three large plexiglass insect rearing cages (600×600×650mm) so that each cage contained ≈ 1333 pupae. The pupae were placed in the open bottom of a large (140mm) petridish which was then placed into a brown paper bag. The paper bags were then placed individually into each of the three cages. The brown paper bags were lightly sprayed with water on a daily basis to create a humid environment which prevents the pupae from potentially desiccating. Cages were checked daily for adult presence.

As soon as the first adults started to emerge, each rearing cage was provided with protein hydrolysate, sugar and water. Adult flies were kept on this diet for ten days to ensure that they were sexually mature before introducing fruit (Papadopoulos et al., 2002). After ten days, 20 fruits of each host were placed into the cages with the flies, in order to have one rearing cage that represents stone fruit, one pome fruit and one citrus. Individual fruits were rinsed in a 2% bleach solution, prior to insertion in the cages, as to sterilize them against unnecessary bacterial- or fungal development. The fruits were then left in the cages for 24h to ensure sufficient eggs to be laid by ovipositing females. After 24h the fruits were removed and each type of host was placed into three new separate plexiglass insect rearing cages (600×600×650mm) on a 3cm layer of sterilized sand (Malmesbury). These fruits were then left to produce the first generation of host specific fruit flies.

In order to remove potential differences in the development of the fruit flies that might have originated as a result of being reared on a diet at CRI, the procedure was repeated with the first generation of host specific fruit flies to produce a second generation of host specific fruit flies. When the second generation of flies emerged as adults, all flies were collected, resulting in 30 males and 35 females from citrus, 21 males and 22 females from pome fruit and 8 males and 7 females from stone fruit. Adults were preserved in specimen jars (40ml) in a

freezer at -12°C and kept until further analysis. The sample size used for pome fruit and stone fruit was small but still fully representative of the usual taxonomic study constraints (Baylac et al., 2003). These individuals were then used to assess developmental differences of being reared on stone fruit, pome fruit and citrus by using geometric morphometrics on the wing structures.

Preparation of wings

The second generation host specific flies were first preserved in 90% ethanol. Flies were then individually processed. In a top view orientation, the right wing of the fly was removed as near to the base of the wing as possible using a pair of super fine no. 5 forceps. The wing was then placed into Xylol to remove surface tension which makes the wing easier to handle. A microscope slide was then prepared with a drop of Entellan® into which the wing was then placed. A cover slide was then carefully put on top of the drop so that the wing was flat between the slide and the cover slide, using the forceps to apply pressure on top as to remove any air pockets. The same was done for the left wing which was mounted onto the same slide in a symmetrical orientation to the right wing. Slides were then clearly marked according to the host fruit, sex and left and right wing. The slides were then left for one day to set.

Imaging and digitizing wings

A photo of each wing was taken using a Leica MZ16A microscope equipped with a DFC290 camera and Leica application suite V4.1 software. All images were captured at a resolution of 2048×1536. All right wing images were digitally flipped to have the same orientation as the left wing images. Fourteen prominent landmarks, twelve that were used by Schutze et al. (2012) with the addition of two landmarks, were digitized on each of the wings (Fig. 4.1)

using tpsDig2 version 2.17 (Rohlf, 2006) and the coordinate data was then exported to MorphoJ version 1.05F (Klingenberg, 2011) for analysis.

Statistical analysis

All wing images were superimposed during a Procrustes fit, after which the outliers were removed. Three outliers were removed due to physical damage of the wing at localities of the prominent landmarks that were used. A principle component analysis (PCA) was done to describe the variation between each of the wings individually. A discriminant function analysis was done to show the degree of separation between different wing shapes of males and females, as well as difference in wing shape of flies reared on different host fruits tested. A Procrustes ANOVA was used to check for differences in size and shape between each individual's left and right wings, as well as for checking differences between the left and right wings among all individuals. A canonical variate analysis (CVA) was performed to check for differences in the shape of each individual fly's wings with respect to the host fruit the fly was reared on. An error file was developed by taking two images of each wing of adult flies (for testing imaging error) from a subset of the data, which were then both digitized (for testing digitizing error). The error file is used to check for irregularities during the imaging process as well as the process of digitizing landmarks on the wings. During geometric morphometrics, shape is defined as all the geometric information about a configuration of landmarks on a certain object, except for size, position and orientation (only shape) (Klingenberg, 2011).

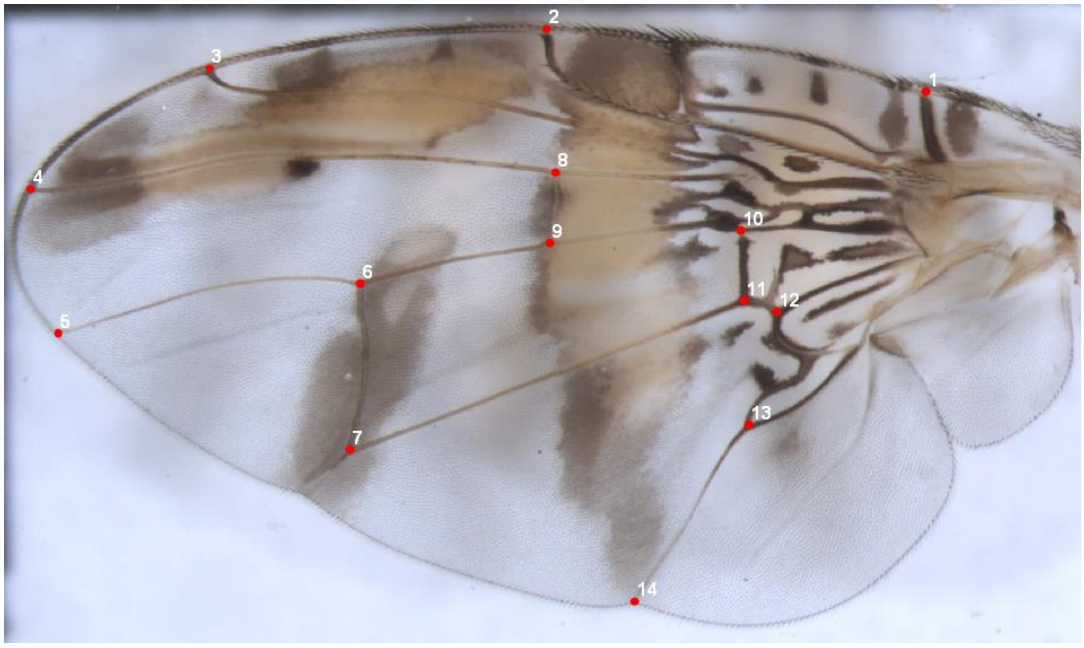

Figure 4.1: Fourteen landmarks that were digitized on wings of *Ceratitis capitata* adults during a geometric morphometric study on the developmental differences of *C. capitata* reared on different host fruits during a laboratory experiment.

Results and discussion

Table 4.1 displays results derived from checking the degree of optical error that might have occurred during the capturing of the images and the degree of error from digitizing the landmarks on the wings.

Table 4.1: ANOVA results for centroid size (information on the size of the landmark configuration), showing imaging and digitizing error for *Ceratitis capitata* wings reared on plums during laboratory experiments.

Effect	SS	MS	df	F	p
Individual	0.08975989	0.0002876919	312	45.43	< 0.0001
Side	0.00017775	0.0000074061	24	1.17	0.2681
Individual*Side	0.00197588	0.0000063329	312	9.15	< 0.0001
Imaging error	0.00046495	0.0000006919	672	1.30	< 0.0001
Digitizing error	0.00071318	0.0000005306	1344		

The mean square (MS) and F values of imaging error and digitizing error are much smaller than the Individual*Side interaction (Table 4.1), indicating that the differences observed in the shape of the individual wings due to error that was caused through imaging and digitizing the wings are negligible. The p values are of less interest in the measurement of error as it is of more relevance to establish by how much the effects of interest exceed the measurement error (Klingenberg & McIntyre, 1998), indicated here by the mean square (MS) of the Individual*Side interaction which exceeds the first error MS by nine times. The imaging equipment and the technique used for digitizing the landmarks on the individual wings were therefore accurate, indicating that the following results were due to developmental factors.

Table 4.2: Effects observed in the differences of wing shape for *Ceratitis capitata* reared on different host fruits during laboratory experiments.

Effect	SS	MS	df	F	p
Individual	0.21942344	0.0000774800	2832	17.42	< 0.001
Side	0.00039112	0.0000162965	24	3.66	< 0.001
Sex	0.13274022	0.0055308426	24	188.06	< 0.001
Fruit	0.00550956	0.0001147824	48	3.90	< 0.001
Individual*Side	0.01259397	0.0000044470	2832		
Fruit*Sex	0.00113360	0.0000236167	48	1.42	0.030
Fruit*Side	0.00020679	0.0000043081	48	0.11	1.00

Significant differences in the shape of wings between males and females were found ($F = 188.06$, $p < 0.001$), indicating sexual dimorphism. Significant differences in the shape of wings from individuals that were reared on different host fruits were found ($F = 3.90$, $p < 0.001$). The effect of side was significant relative to the Individual*Side interaction, shown by the four fold increase in MS values. This indicates that there is directional asymmetry (systematic difference between left and right wings) (Table 4.2). The Fruit*Sex interaction was found to be significant ($F = 1.42$, $p = 0.030$). This interaction reflects the high significance that was found for the differences in wing shapes between males and females, but across the different host fruits. The Fruit*Side interaction was not significant indicating that the host fruit did not have an effect on the differences between the left and right wings of individual flies.

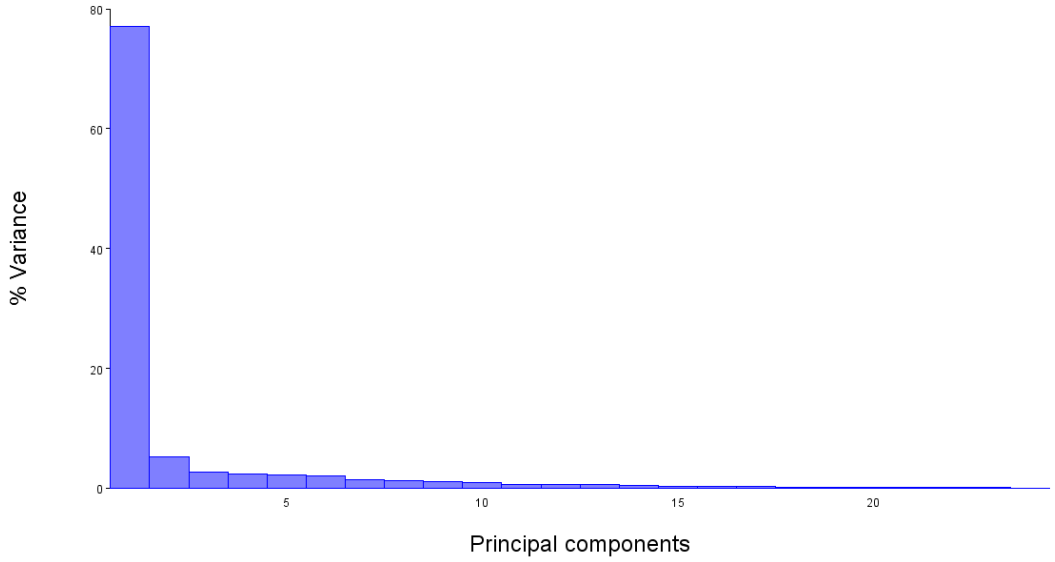

Figure 4.2: A principle component analysis (PCA) displaying the variation in wing shape of *Ceratitis capitata* males and females during laboratory experiments.

The PCA indicates that nearly 80% of all variation observed in the wing shapes of adult *C. capitata* can be explained by the first principle component (Fig. 4.2). By looking at the variation of developmental processes distinct patterns can be generated of multiple morphological traits that are affected by the development process (Klingenburg & McIntyre, 1998).

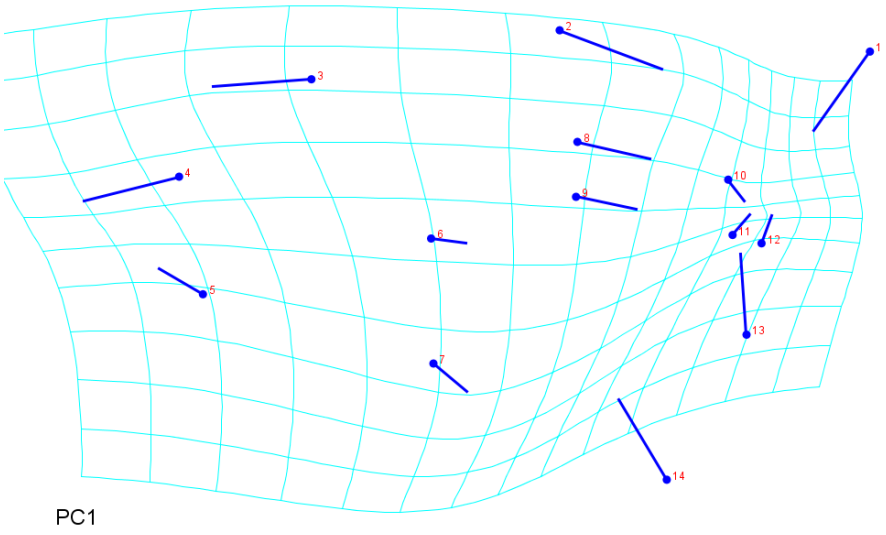

Figure 4.3: Principle component 1 (PC1) transformation grid showing 80% of the total variation that occurred in wing shape observed in *Ceratitis capitata* males and females during laboratory experiments. Dots represent the average shape and the line represents the shift of the landmark associated with PC1 on a scale factor of 0.3.

Landmarks 1, 2, 3, 4, 5, 7 and 14 outline the wing (Fig. 4.3). PC1 indicates that an elongation of the wing occurred (landmarks 3, 4 and 5), with a narrowing of the base of the wing (landmarks 1 and 14), relative to the average shape of all wings. Changes in the wing shapes were observed but can not, at this point, be related to any behavioural issue relating to flies breeding from different fruits.

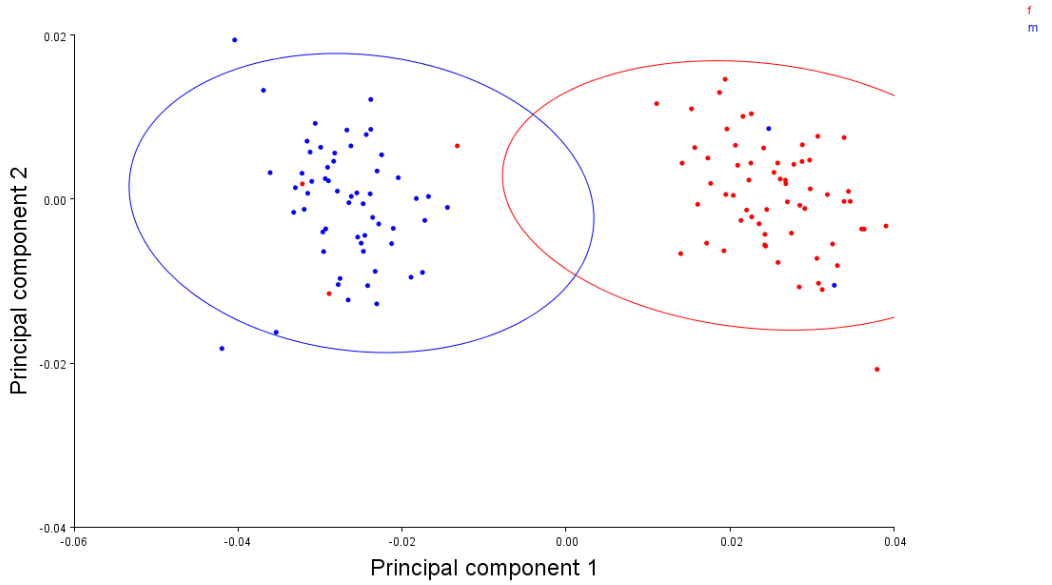

Figure 4.4: The main variation in the shape of all individual wings of *Ceratitis capitata* males (m) and females (f) plotted as a single point with (x;y) coordinates during laboratory experiments conducted.

Two clear groupings of wing shapes can be seen for male and female *C. capitata* (Fig. 4.4) when plotting the variation in the shape of the wings, explained by PC1 (x-axis) and PC2 (y-axis), as (x;y) coordinates. This indicates that the 80% variation in wing shape that could be explained by the first principle component, observed in the PCA (Fig. 4.2), is mainly due to the differences in wing shape among the males and females of *C. capitata*. For this reason, it was necessary to separate males and females for any further analyses. Esterhuizen et al. (2014) describe sex as a major factor that influences the phenotypic plasticity of *C. capitata* at different acclimation temperatures during the development of these flies.

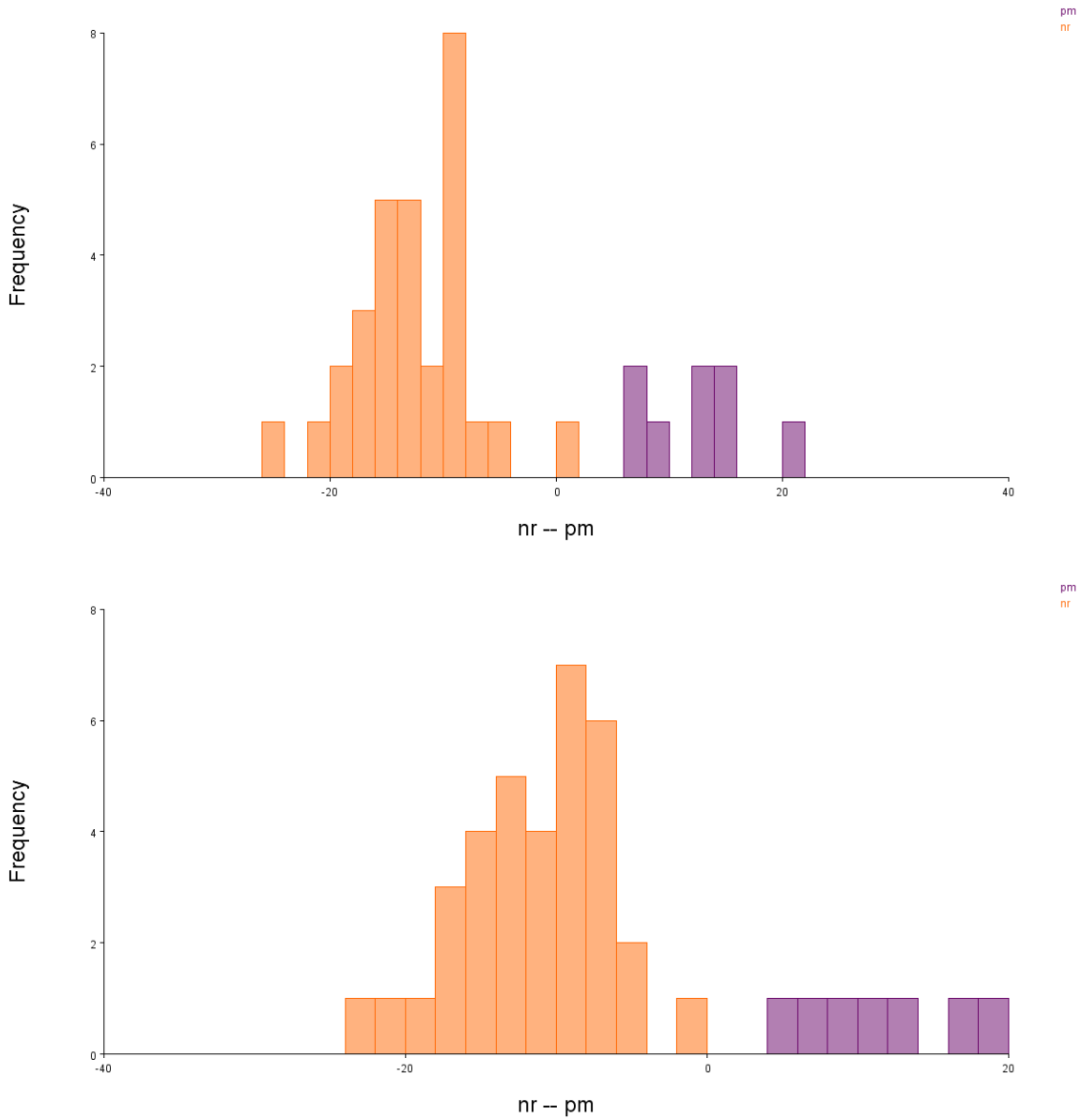

Figure 4.5: Discriminant function analysis displaying the degree of separation between the wing shape of *Ceratitis capitata* males (top) and females (bottom) reared on citrus (nr) and stone fruit (pm) during a laboratory experiment.

A clear, significant separation of wing shapes can be seen between *C. capitata* reared on citrus and stone fruit in both females (T-square = 133.013, p = 0.037) and males (T-square = 161.344, p = 0.049) (Fig. 4.5). Only one out of 30 males that were reared on citrus displayed a wing shape that was more associated with that of flies reared on stone fruit.

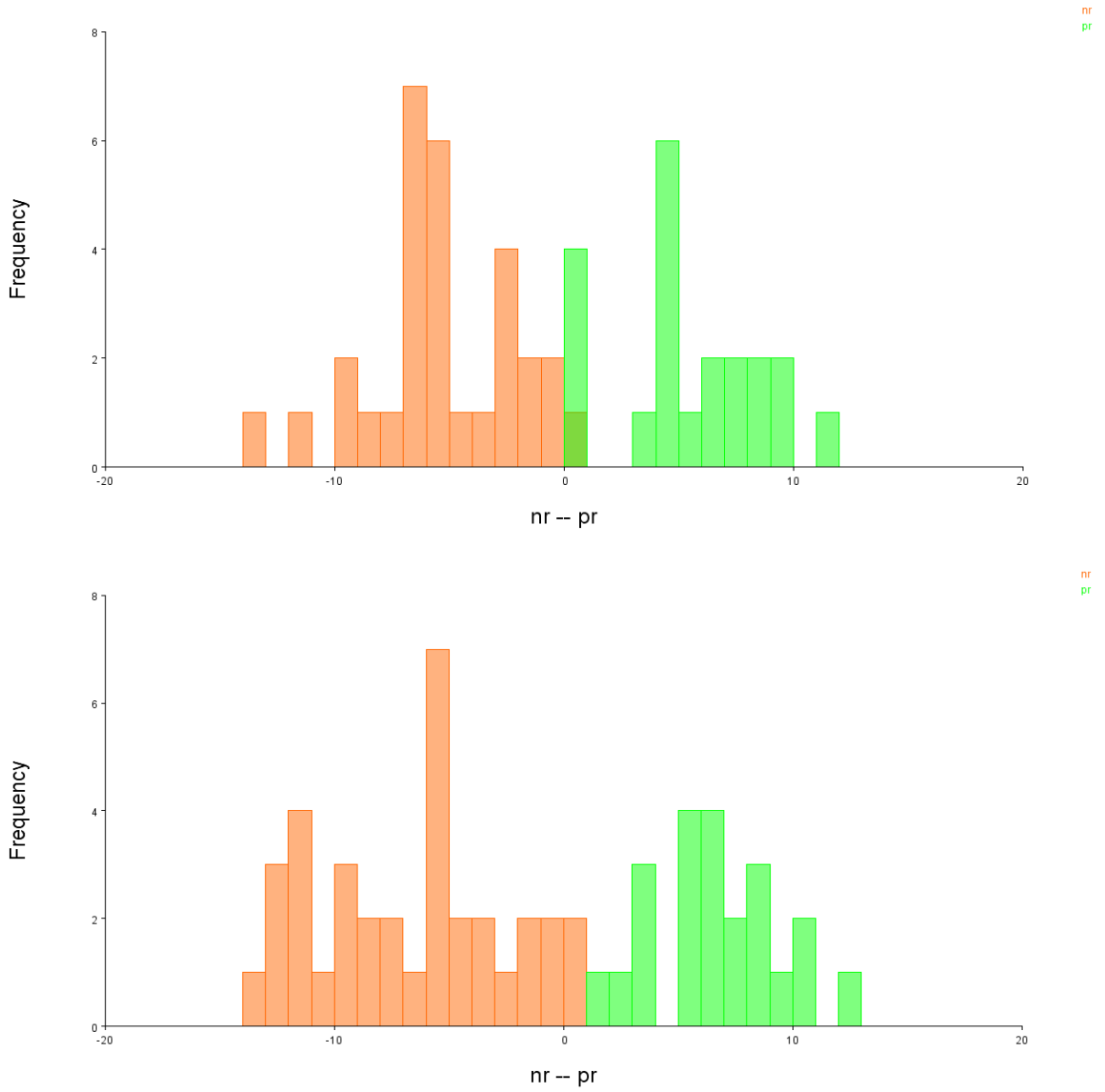

Figure 4.6: Discriminant function analysis displaying the degree of separation between the wing shapes of *Ceratitis capitata* males (top) and females (bottom) reared on citrus (nr) and pome fruit (pr) during a laboratory experiment.

When reared on citrus or pome fruit, a clear, significant separation of wing shapes can also be seen between *C. capitata* females (T-square = 180.693, $p < 0.001$) and males (T-square = 132.774, $p = 0.004$) (Fig. 4.6). Two flies out of 35 females that were reared on citrus displayed wing shapes that were more associated with that of flies reared on pome fruit, while one male out of 30 that was reared on citrus displayed a wing shape that was more associated with that of flies reared on pome fruit.

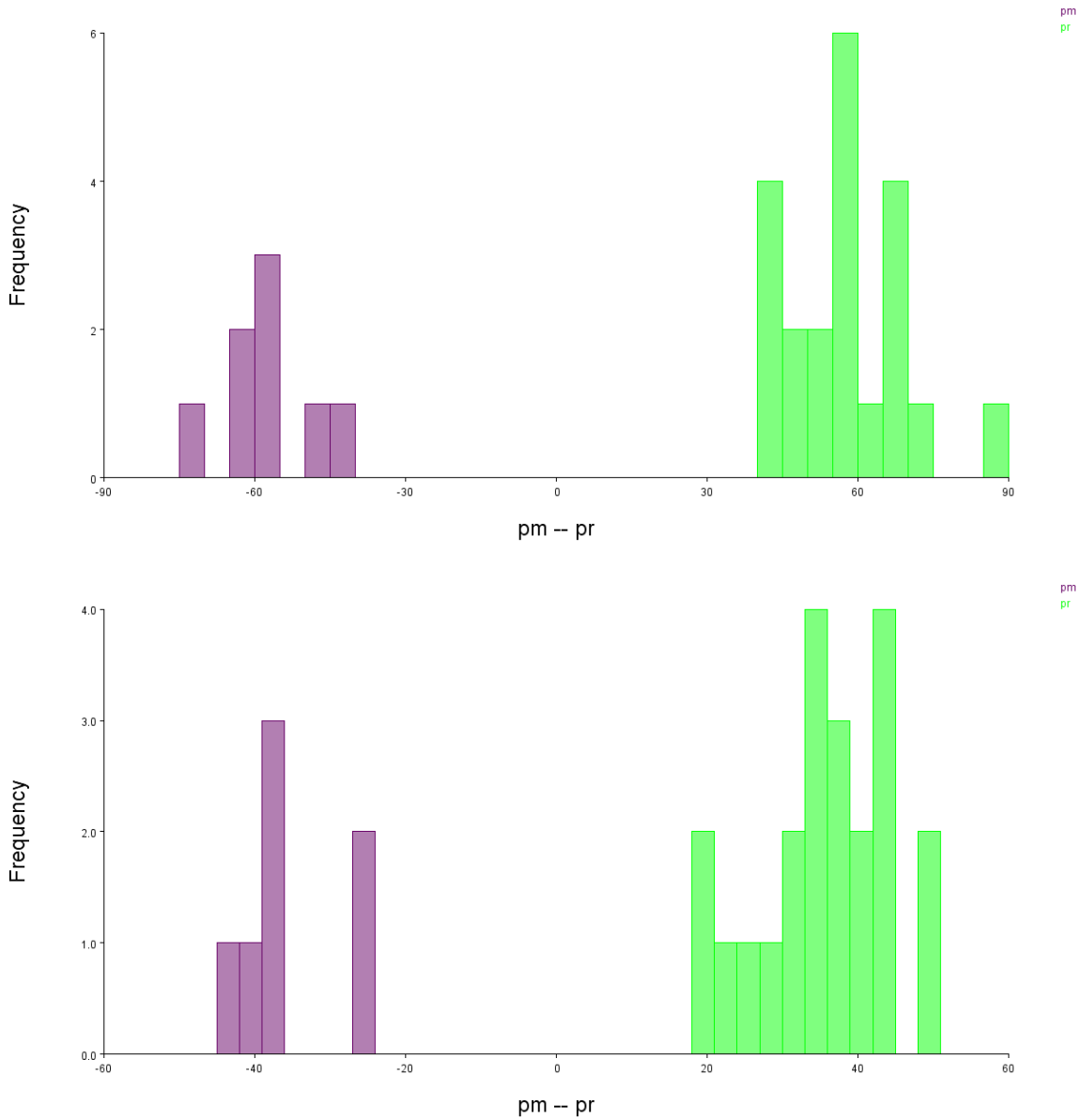

Figure 4.7: Discriminant function analysis displaying the degree of separation between the wing shapes of *Ceratitis capitata* males (top) and females (bottom) reared on stone fruit (pm) and pome fruit (pr) during a laboratory experiment.

A clear, but statistically non-significant, separation of wing shapes can be seen between *C. capitata* females (T-square = 376.591, p = 0.215) and males (T-square = 662.079, p = 0.090) that were reared on stone fruit and pome fruit (Fig. 4.7). All wing shapes in these groups are associated with the respective host fruit on which individual flies were reared.

Based on the discriminant function analysis, the most pronounced difference was that between stone and pome fruit, showing the highest T-values (Fig. 4.7). However, the results

were found to be non significant, which would be due the relatively small sample size (relative to the number of landmarks), resulting from few flies having developed from pome fruit. For this reason the good separation obtained here cannot be reliably classified and caution should be made with intepretation. More data would be needed to confirm these comparisons.

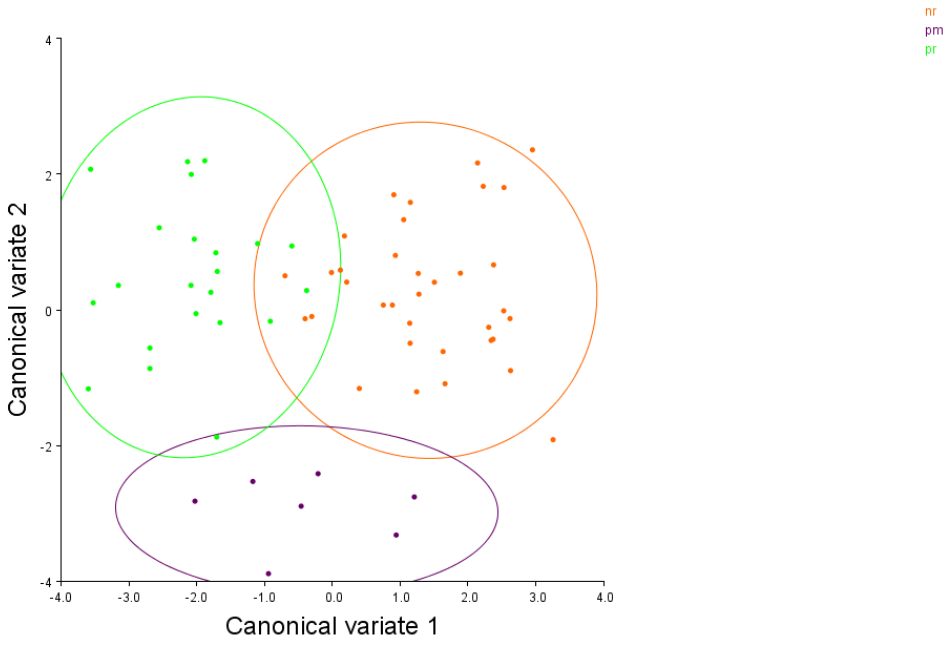

Figure 4.8: Differences in the shape of female *Ceratitis capitata* wings that were reared on different host fruits during laboratory experiments. Citrus (nr), stone fruit (pm) and pome fruit (pr). Confidence ellipses denote 95% probability.

Table 4.3: The p-values of a canonical variate analysis (CVA) done on the Procrustes distances among female *Ceratitis capitata* wings. Females were reared on different host fruits during laboratory experiments.

	Citrus	**Stone Fruit**
Stone Fruit	0.210	
Pome Fruit	0.110	0.144

There were non-significant differences between Procrustes distances (square root of the sum of squared distance between corresponding landmarks of two configurations) of wing shapes observed for female *C. capitata* that were reared on citrus (clementines), stone fruit (plums) and pome fruit (pears) (Table 4.3).

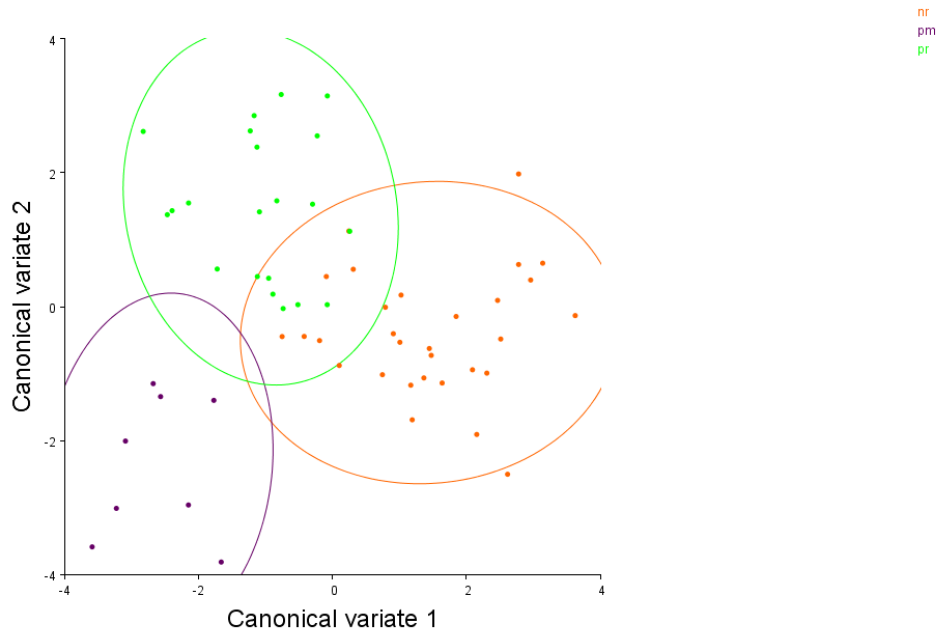

Figure 4.9: Differences in the shape of male *Ceratitis capitata* wings that were reared on different host fruits during laboratory experiments. Citrus (nr), stone fruit (pm) and pome fruit (pr). Confidence ellipses denote 95% probability.

Table 4.4: The p-values of a canonical variate analysis (CVA) done on the Procrustes distances among male *Ceratitis capitata* wings. Males were reared on different host fruits during laboratory experiments.

	Citrus	**Stone Fruit**
Stone Fruit	0.052	
Pome Fruit	0.093	0.002

The differences in Procrustes distances between corresponding landmarks in the wings of male *C. capitata* flies were highly significant (p = 0.002) between individuals that were reared on stone fruit (plums) and pome fruit (pears), and non-significant for individuals

reared on stone fruit (plums) and citrus (clementines) (p = 0.052) and pome fruit (pears) and citrus (clementines) (p = 0.093) (Table 4.4). This result mirrors that of the discriminant function analysis, which compares wing shape, and also finds strong differences between pome and stone fruit. The canonical variate analysis is an extension of the discriminant function analysis in that the goal here is to see the pattern of variation across all groups, which would explain why no significance was found for females. This method does not explain whether wings are larger or smaller, it purely indicates that there are differences in the shape of fruit fly wings that developed on different hosts. The geometric morphometric method, applied here, eliminates size effects as well as positional and orientation effects and leaves only shape.

The differences observed above could be due to the difference in the nutritive value of the host fruits and be representative of the suitability of the type of fruit for the development of male *C. capitata* flies. Yeap et al. (2013) found differences in size and wing shape of laboratory reared *Aedes aegypti* mosquitoes compared to individuals in the wild population and stated that these differences were mainly attributed to nutrition. This significant difference in the Procrustes distances could have also been caused by the type of post-harvest treatment of the fruits or the pesticides that were used to spray the fruit in order to prevent attack from these pests. The clear grouping of adult flies into three groups (Fig. 4.8 and Fig. 4.9) does raise more questions on whether it is the fruit type that influences the difference in the shape of the wings, and whether this method could be used to indicate which fruit types are more favourable for the optimal development of these flies. The females appeared to be less susceptible to wing changes on different hosts than the males. It can be hypothesized that the wings of male *C. capitata* are a more plastic trait compared to females of this species, as males rely on the size and shape their wings to fan sex pheromone to females as well as signalling visual and acoustic stimuli in order to successfully copulate (Prokopy &

Hendrichs, 1979). Differences in male fitness exist, if fitness is viewed as the event of successful copulation, otherwise all males would have been equally successful in mating which is not the case (Prokopy & Hendrichs, 1979).

Conclusion

The results of the present study do not conclusively explain why differences in the shapes of *C. capitata* wings were observed, but it becomes apparent that this method could be used to reveal developmental traits associated with different types of host plants. Morphometric studies that want to determine shape differences between species, should take into account the influence that the host has on the morphology of the species. A greater sample size over a wider range of host plants is recommended to reveal patterns of development associated with these hosts. The quality of fruits and vegetables are affected by long term cold storage (Johnston et al., 2002) and for this reason future experiments should be done on hosts that are acquired in their ripe stage and that are in a near natural status where they have not been exposed to any pesticides, as residues on hosts acquired from markets could affect results. Further studies with a greater sample size would have to be conducted to confirm shape differences of flies developing on pome fruit in particular, as the sample size in this study was not large enough to reliably make group comparisons.

Future studies on development effects that fruit flies experience when reared on different host plants, by using geometric morphometrics as a tool for looking at variations, should include a reference host that is known for optimal development of the species under question so that the results of other host plants can be compared to this reference fruit. When those results are then compared in relation to the reference fruit it can be assumed that the closer the grouping occurs to the reference fruit grouping, the more optimal a host is for the development of the

specific fruit fly species and vice versa. Guava, for example, was found to have the highest infestation levels and the highest number of pupae that emerged per fruit and per kg fruit during laboratory experiments done on wild collected fruit (José et al., 2013). Mwatawala et al. (2009) also found that guava species had some of the highest infestation levels and that guava is a preferred host of certain tephritids. In future studies, traditional morphometric measurements, geometric morphometrics and flight studies should be used in an integrated fashion, to assess the effects of the host fruits on wing shape variation more comprehensively.

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Chapter 5: General conclusion

Ceratitis capitata and *C. rosa* were observed in various developmental, relative abundance and distribution studies in the Western Cape. *C. capitata* was found to be the predominant fruit fly pest in the Western Cape. The highest trapping of *C. rosa* (N = 1940) occurred in the Stellenbosch region, but still remained well below the number of *C. capitata* collected (N = 2893). In the other regions sampled total *C. rosa* numbers remained relatively low throughout the two-year survey period. The high numbers of *C. capitata* collected, and the fact that they were mostly all males, could be due to the survey that was done in regions where the Sterile Insect Technique (SIT) is implemented. The most effective attractants for luring and trapping *C. capitata* and *C. rosa* were observed to be BioLure® (Chempac (Pty) Ltd, Paarl) and PheroLure™ (Insect Science™, Tzaneen, Limpopo Province, South Africa) (an enriched ginger root oil (EGO)). It is recommended that these two attractants be used during similar studies, for *C. capitata* and *C. rosa* monitoring or detection programmes. During a study to compare the competitiveness of fertilizers with proteinaceous baits, Mazor (2009) found that *C. capitata* females were most attracted to pelletized poultry manure (45.9% of N = 200) followed by crystalline ammonium acetate (38.57% of N = 200), the key component of dry bait BioLure®. EGO lure was found to attract 96% of all (N = 3824) *Ceratitis* males sampled during a survey of African *Ceratitis* species, and as a result has been recommended for the monitoring and trapping of *Ceratitis* species (Mwatawala et al., 2012).

Field collected fruits that were favoured most by *C. rosa* and had the highest infestation indices were guavas (*Psidium guajava* L.) (106.089 adults/kg) and jambos, also known as rose apples (*Syzygium jambos* (L.) Alston) (280.543 adults/kg). The field collected fruits

favoured most by *C. capitata* were pears (*Pyrus communis* L.) (53.306 adults/kg), peaches (*Prunus persica* Sieb. & Zucc.) (54.677 adults/kg) and piquanté peppers (*Capsicum baccatum* L.) (57.036 adults/kg). Myburgh (1956) reported peaches to be the most favoured host by *C. capitata* and *C. rosa*, and that pears were adequate hosts for the development of the two species. He described guava as an important alternate host for both species of fruit fly due to the rapid development they experience on guava. In a list of world host species of *C. capitata* compiled in Myburgh (1956), members of the family Solanaceae (peppers) include sweet green (*Capsicum annuum* L.), oxheart pepper (*Capsicum annuum* L. var. *cerasiforme* Mill.), cherry pepper (*Capsicum annuum* L. var. *conoides* Mill.), bell pepper (*Capsicum annuum* L. var. *grossum* Sendt.) and bush red pepper (*Capsicum frutescens* L.). The infestation of piquanté peppers by *C. capitata* has not yet been reported for the Western Cape, and should be considered an important alternate host of *C. capitata*, and potentially *C. rosa*, due to the high level of infestation found in the present study. It is thus important to focus management of *C. capitata* and *C. rosa* on areas where these host fruits occur in the Western Cape, whether on a commercial or non-commercial scale.

Results acquired from the laboratory developmental studies of *C. capitata* and *C. rosa*, on various commercial deciduous host fruits, are in line with field collected data. The developmental success for both species of fruit flies were found to be the highest on guavas with significantly higher numbers of larvae emerging to form pupae and successfully develop into adults, compared to other host fruits tested. The adults that emerged from pupae that were reared on guava also displayed higher fecundity rates, in terms of eggs laid per female per day, and better overall fitness as these adults had the longest lifespan (> 137 days for *C. rosa* and > 148 days for *C. capitata*) compared to adults that emerged from grapes. These results are in line with findings of Myburgh (1956) on increased development of both

Ceratitis species observed on guavas, in terms of the time required for egg and larval development, during laboratory experiments.

It should be noted that the artificial inoculation of eggs, as done in chapter three, may not be suitable for laboratory experiments to determine host suitability (life table parameter studies) for the development of fruit fly pests. In the present study both *C. capitata* and *C. rosa* did not naturally infest citrus (oranges) during the egg hatch experiment. Both species did however develop, to some degree, in clementines during the artificial inoculation experiment. During the rearing phase of the flies used in the geometric morphometrics chapter, *C. capitata* were most successful on clementines, compared to pears and plums. This could be explained by differential resistance of citrus varieties to *C. capitata* attack (Rössler & Greany, 1990). The peel oil content and peel puncture resistance are affected by senescence and play a role in the fruits resistance to fruit fly attack. Papachristos et al. (2008) found that the pulp of citrus fruit is favourable for the development of *C. capitata* immature stages, but that the rind causes mortality. The female ovipositor is also more likely to only reach the flavedo and albedo regions of the citrus fruit, in which the eggs will be laid, and it is then up to the larvae to bore through these regions into the pulp in order to successfully develop (Papachristos et al., 2008). The fact that the flies during the rearing stage of the geometric morphometrics experiment, of the present study, did so well could be attributed to the thin peel of the clementines that were used.

Geometric morphometrics revealed clear groupings in the wing shapes of *C. capitata* that were reared on different commercial hosts. The results in the present study do not explain why these variations in wing shape occurred, but that this method holds potential for determining slight variations in wing shapes of *C. capitata* flies that are brought on by the quality of the host plant in which they have developed.

Due to the restraints of experiments that are conducted under laboratory conditions, where it is difficult to fully replicate the natural environment in which these flies would usually occur, these developmental and life table parameters should be interpreted with some caution. It is, however, a first step towards determining such parameters for these species and to invoke a better understanding of why these species select certain host plants more than others in their natural environment and habitat. This will in turn lead to a better understanding of why there are differences in the spatial distribution of these two fruit fly species. Apart from host plants being a determining factor for the relative abundance and distribution of these species, it is important to also focus on abiotic factors that may influence their distribution, such as rainfall (Vargas et al., 1983; Vayssières et al., 2009; De Villiers et al., 2013), temperature (Myburgh, 1956) and elevation (Israely et al., 2005).

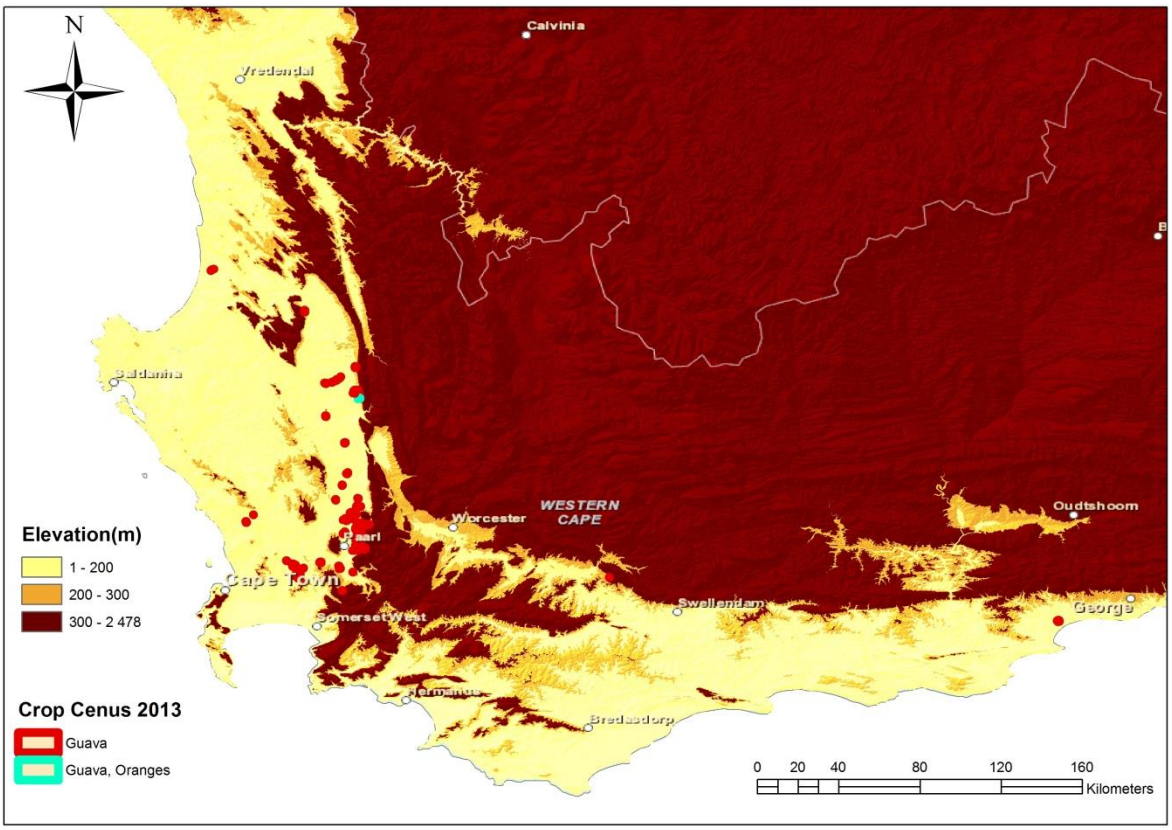

Figure 5.1: Main commercial guava stands in the Western Cape, South Africa. Sampling sites are marked as Stellenbosch (SB), Elgin (EL), Villiersdorp (VD) and Worcester (WO). (Western Cape Department of Agriculture (WCDoA) Aerial Census Data, 2013).

In the present study *C. rosa* abundances were observed to be higher at lower elevations between 78.5m and 319m (Fig. 2.6). In Fig. 5.1, most of the commercial guava stands in the Western Cape occur within this altitudinal range. Guava stands can therefore contribute to the high abundances of *C. rosa* in Stellenbosch, as it is a favoured host that is located closer to Stellenbosch compared to the other sampling sites.

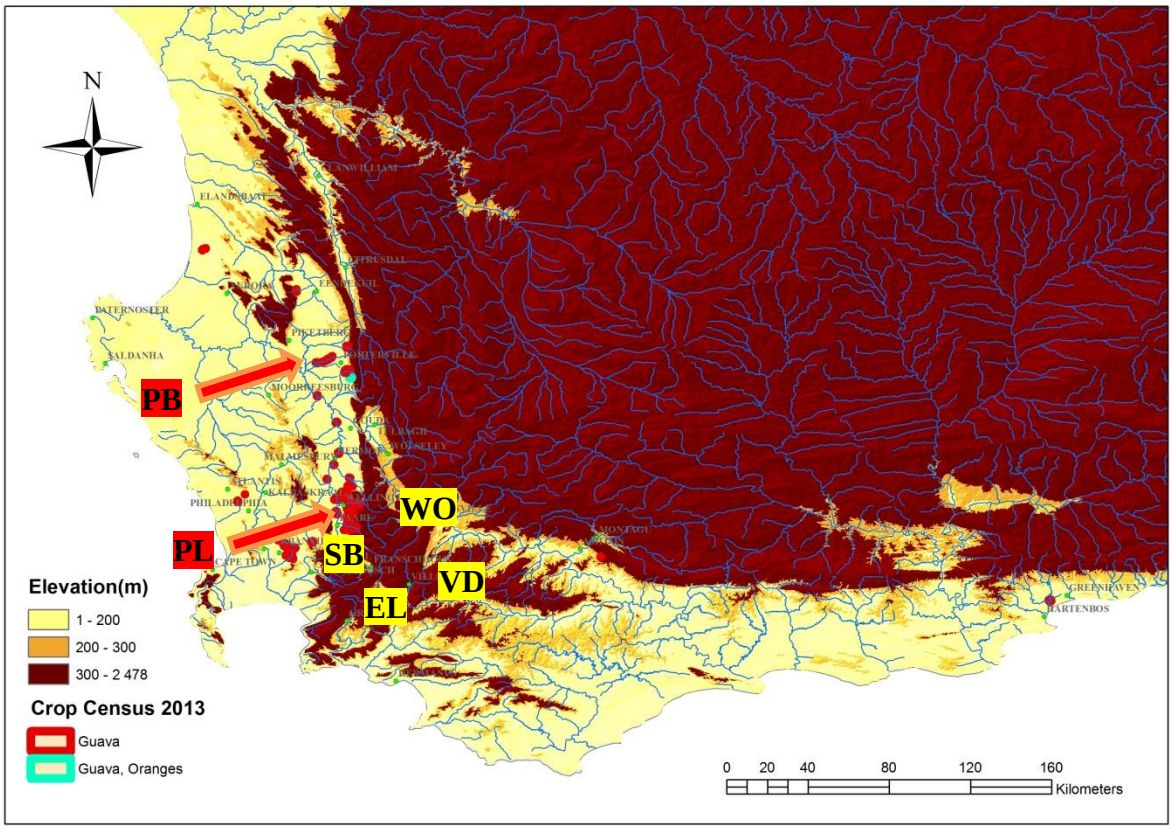

Figure 5.2: Main commercial guava stands, Piketberg (PB) and Paarl (PL), and rivers of the Western Cape, South Africa. Sampling sites are marked as Stellenbosch (SB), Elgin (EL), Villiersdorp (VD) and Worcester (WO). (Western Cape Department of Agriculture (WCDoA) Aerial Census Data, 2013).

Vargas et al. (1983) found that when abundances of *C. capitata* were higher at the head of a valley, then the abundances were higher at the mouth of the valley. They describe that field observations of *C. capitata* suggest that the flies move along vegetation along rivers and that

tributaries, narrow canyons and valley mouths funnel the flies to other locations. The valleys, and rivers running through the areas of guava production in the Western Cape, could serve to funnel *C. rosa* and cause quicker dispersal of the species into areas where they are not as prevalent. The main stands of commercially grown guava occur in the Paarl and Piketberg areas, with smaller stands of guava located along the Berg river (Fig. 5.2). Paarl is situated in the upper catchment area of the Berg river from where it runs towards Piketberg and then discharges into the Atlantic Ocean near Velddrif. Taking the findings of Vargas et al. (1983) and De Villiers et al. (2013) into account, the Berg river holds the potential to facilitate the distribution of *C. rosa* into northern parts of the Western Cape, along the West coast.

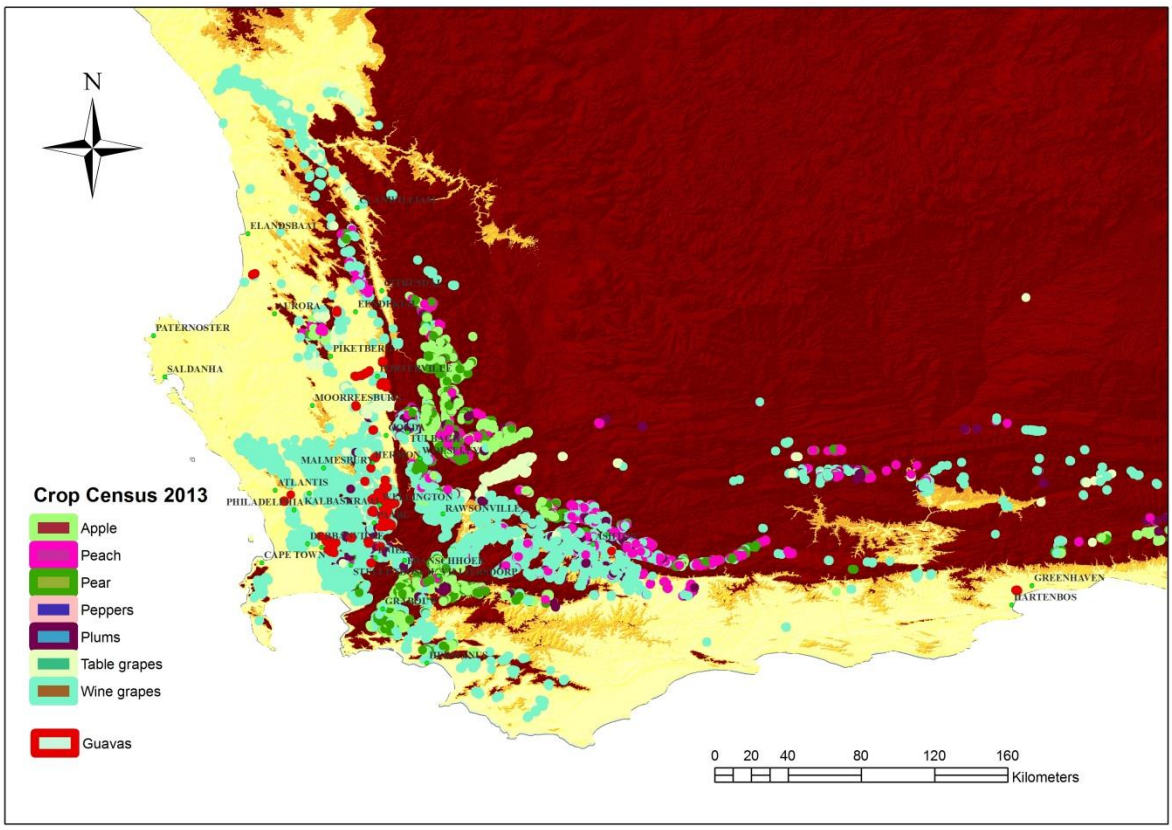

Figure 5.3: Main stands of commercially grown deciduous fruits and the main commercial guava stands of the Western Cape, South Africa Western Cape Department of Agriculture (WCDoA) Aerial Census Data, 2013).

Main commercial guava stands are situated in an almost central position relative to wine grape crops in the Western Cape (Fig. 5.3). Further research is required in the areas where guavas are commercially grown, in order to investigate whether *C. rosa* and *C. capitata* abundances are higher in these areas compared to areas where other deciduous fruits such as apples and pears are grown, but taking abiotic factors such as elevation into account. With current mapping software, such as ArcGIS® 10 (Esri®, Redlands, California, 2010), it is possible to first establish the main crops for a certain area, on which preliminary laboratory experiments could be done in order to predict the acceptability of the host plants grown in that area to the specific pest species occurring in that area. This could prove a quicker and more cost effective approach to establish potential hotspots for the pest species under consideration. If follow-up monitoring through trapping data and fruit damage assessments corresponds to the preliminary laboratory experiments, the area should be proposed to be included in the area-wide management of the particular pest species.

According to the main findings in the present study, it is recommended that fruit fly management efforts in the Western Cape, should include areas where guavas are commercially produced (Fig. 6.3). SIT and BAT is currently implemented only in regions located on the Eastern side of the Hottentots Holland mountain range (which include main areas producing table grapes, a suitable host for both fly species as determined from the present study), while guavas are produced mostly to the West of this range. According to Karsten et al. (2013), fruit flies are dispersed throughout South Africa, mostly via human-mediated dispersal, based on their genetic population structure. Due to this, areas of high pest pressure can contaminate other areas with ease. It remains equally important to survey home gardens and farm gardens, near or in these agricultural areas, with the aim to establish a census on important alternate hosts such as guavas, jambos and piqaunté peppers for such

gardens. The findings in this study should be incorporated into the area-wide integrated pest management programmes for the Western Cape Province, South Africa.

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